"I have dreamed in my life, dreams that have stayed with me ever after. They have gone through and through me, like wine through water, and altered the color of my mind.

-Emily Brönte

University of Alberta

Proton MRS Investigation of Glutamate and Executive Functioning in First Episode Psychosis

by

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Abstract

Glutamatergic dysfunction has been reported in schizophrenia, particularly in the region of the prefrontal cortex. Likewise, frontal lobe cognitive deficits, especially in the domain of executive functioning, are present at the first episode of illness and predict functional outcome. It is unclear how glutamate may relate to the observed executive dysfunction in this illness. The current study examines the glutamate system of the medial frontal cortex *in vivo* in first episode psychosis and its possible relationship to executive functioning.

Individuals with a first episode of psychosis and healthy volunteers underwent one 3T ^{1}H-MRS scan of the glutamate system and neuropsychological testing of executive function. Patients had lower glutamate levels and poorer performance on the executive tests. However, executive performance was not correlated with glutamate levels in either group. These results support a hypoglutamatergic model of the illness, but do not provide additional evidence linking glutamate to executive functioning.

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Dedication

I would like to dedicate this manuscript to those individuals who have sparked my dreams and ignited my imagination – angels, real and disguised, who have come to my aid more times than I can count.

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List of Abbreviations

AC	Anterior cingulate
ACC	Anterior cingulate cortex
Ach	Acetylcholine
Acetyl Co-A	Acetyl coenzyme-A
ADL	Activities of daily living
α-KG	alpha ketoglutarate
ATP	Adenosine triphosphate
BA	Broadmann's area
BAI	Beck Anxiety Inventory
BDI	Beck Depression Inventory
BRMS	Bech-Rafaelsen Mania Scale
Ca^{2+}	Calcium ion
Cho	Choline
CNS	Central nervous system
Cre	Creatine
CT	Computed tomography
DA	Dopamine
DLPFC	Dorsolateral prefrontal cortex
DRN	Dorsal raphé nucleus
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th ed.
EAAT	Excitatory amino acid transporters
EEPIC	Edmonton Early Psychosis Intervention Clinic

FEP	First episode psychosis
fMRI	Functional magnetic resonance imaging
GABA	gamma aminobutyric acid
GAD	Glutamic acid decarboxylase
GAF	Global Assessment of Functioning
GCP II	Glutamate carboxypeptidase II
GDH	Glutamate dehydrogenase
Gln	Glutamine
Glu	Glutamate
Glx	Glutamate + Glutamine + GABA
GM	Grey matter
GS	Glutamine synthetase
GT	Glutamate transporter
HC	Healthy control
HCT	Halstead Category Test
^{1}H-MRS	Proton magnetic resonance spectroscopy
^{1}H-MRSI	Proton magnetic resonance spectroscopic imaging
HST	Hayling Sentence Completion Test
5-HT	5-hydroxy tryptamine (serotonin)
K^{+}	Potassium ion
kg	kilogram
LTP	Long term potentiation
Mg^{2+}	Magnesium ion

MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
μM	micromolar
mmol	millimolar
mPFC	Medial prefrontal cortex
Na^+	Sodium ion
NA	Nucleus accumbens
NAA	N-acetylaspartate
NAAG	N-acetylaspartylglutamate
NAALADase	N-acetyl-α-linked acidic dipeptidase
NMR	Nuclear Magnetic Resonance
NPT	Neuropsychological tests
OFC	Orbitofrontal cortex
PAG	Phosphate activated glutaminase
PANSS	Positive and Negative Syndrome Scale
PCP	Phencyclidine
PDE	Phosphodiesters
PET	Positron emission tomography
Pi	Inorganic phosphate
PME	Phosphomonoesters
ppm	Parts per million
PPVT-III	Peabody Picture Vocabulart Test – Third Edition
RF	Radiofrequency

SCID	Structured Clinical Interview for the DSM-IV
SN	Substantia nigra
STG	Superior temporal gyrus
TE	Echo time
T_{inv1}	1st Inversion time
T_{inv2}	2nd Inversion time
TM	Mixing time
TR	Repetition time
VBR	Ventricle-to-brain ratio
VOI	Volume of interest
vPFC	Ventromedial prefrontal cortex
VTA	Ventral tegmental area
WAIS-III	Wechsler Adult Intelligence Scale – Third Edition
WBV	Whole brain volume
WCST	Wisconsin Card Sorting Test
WM	White matter
Y-BOCS	Yale-Brown Obsessive Compulsive Scale
zexe	Executive z-score
Zn^{2+}	Zinc

A. Introduction

Schizophrenia is a severe and chronic psychiatric illness characterized by positive and negative symptoms, mood symptoms, and cognitive deficits (Flashman and Green, 2004). The cognitive deficits seen in this illness, including attention and executive skills, predict functional outcome in patients more reliably than clinical symptoms, and are present early in the illness (Green, 1996; Green et al., 2000; Velligan et al., 1997). Understanding the neurochemical substrates of cognitive dysfunction has become increasingly important as future development of pharmacological treatments in schizophrenia will attempt to improve these core cognitive deficits (Green et al., 2004). Recently, various lines of evidence have implicated glutamatergic dysfunction in schizophrenia, particularly in the region of the prefrontal cortex (reviewed in Goff and Coyle, 2001), but how these abnormalities relate to the observed frontal lobe cognitive deficits such as executive function is not clearly understood. The current study examines the glutamate system of the medial frontal cortex *in vivo* in first episode psychosis and its possible relationship to executive functioning.

Chapter 1

Schizophrenia: Background

Classification

Of the psychiatric illnesses, none has provoked more curiosity and misunderstanding than the more chronic of these, schizophrenia. Eugene Bleuler (1857-1940) coined the term *schizophrenia* replacing Emil Kraepelin's (1856-1926) earlier term of *dementia praecox,* which had emphasized the early onset and purported

deteriorating cognitive course of the illness (Sadock et al., 2003). Bleuler's reconceptualization is derived from the Greek roots *schizo* meaning *split* and *phrene* meaning *mind*. Individuals with the disorder experience a "split from reality" and have difficulty distinguishing reality from fantasy, a clinical state known as psychosis. These early psychiatrists helped establish methods for classifying the symptomatology in schizophrenia and importantly, recognized the fragmenting of cognitive processes as a primary defining feature of the illness (Andreasen, 2000).

Schizophrenia affects approximately 1% of the world's population (Weiser et al., 2005), and relative risk for the disorder is known to increase with familial loading (Tsuang, 2000). Although etiology still remains unclear, schizophrenia is believed to result from a complex interaction of numerous susceptibility genes and environmental factors (Harrison and Owen, 2003). For example, monozygotic twins sharing 100% of their genetic material have a 40-60% concordance rate, suggesting an environmental component that is not clearly understood (Sadock et al., 2003). Possible environmental factors suggested to play a role in the development of schizophrenia include gestational and birth complications, exposure to influenza epidemics, maternal starvation during pregnancy, Rhesus factor incompatibility, winter/ spring births, and urban environments (Torrey et al., 1997; Marcelis et al., 1998; Jones and Cannon, 1998; Miyamoto et al., 2003). These epigenetic factors are considered environmental stressors conferring "multiple hits" on an already vulnerable system, leading to emergence of the disorder (Jones and Cannon, 1998; Andreasen, 2000).

While schizophrenia can develop in both men and women, it occurs more often in males who are more severely affected by the illness (Andreasen, 2000). Men

typically have an earlier age of onset ranging from late teens to early adulthood. Onset for women displays a bimodal distribution with the first peak emerging in the mid-twenties, and a second peak in middle age (Sadock et al., 2003). Suicide is still the leading cause of mortality in individuals with schizophrenia, with estimates ranging from 10-15% of all patients with the illness completing suicide (Sadock et al., 2003).

According to formal diagnostic classification, the illness is defined as having two or more of the following active phase symptoms for at least one month: delusions, hallucinations, disorganized speech (e.g. derailment, incoherence), grossly disorganized or catatonic behavior, and negative symptoms (e.g. alogia, avolition) [Diagnostic and Statistical Manual of Mental Disorders, 4th edition, (DSM-IV)]. To meet full DSM-IV criteria for the disorder, illness duration must be at least six months (with one month of active symptoms as described above), accompanied by significant social or occupational dysfunction. Exclusionary diagnoses include disturbances due to a mood disorder, schizoaffective disorder, pervasive developmental disorder, substance abuse, or a general medical condition (DSM-IV).

Symptom Clusters

The illness has been conceptualized in numerous ways in order to account for the core clinical symptoms. The traditional approach has been to divide symptoms into *positive* and *negative* categories. The former term is used to describe features of the illness that have been *added* in individuals with the disorder. These symptoms include delusions, hallucinations, and disorganized speech and behaviour. Disordered thought is believed to underlie the delusional and disorganized symptoms of the

illness. Disorders of thought can be divided into problems with thought content, form of thought, and thought process (Sadock et al., 2003). Delusions, a disorder of thought content, are false personal beliefs based on erroneous inferences about reality. They can assume different themes such as somatic, paranoid (e.g. persecutory, delusions of reference), or patently bizarre. Disorganized speech reflects disorders in the form of thought, including looseness of associations, flight of ideas, and neologisms (inventing new words). Disorders in thought process also include delusional beliefs of thought broadcasting and thought withdrawal (Sadock et al., 2003).

Hallucinations are perceptual experiences with no basis in reality. These can occur in any of the five sensory modalities, but the most common type of hallucination is auditory. Auditory hallucinations often include voices that may be derogatory, commanding, or commentating on the patient's behavior (Sadock et al., 2003).

Negative symptoms reflect features that have been *removed* or are missing in individuals with the illness. These include, among others, anhedonia (inability to experience pleasure), avolition (absence of goal-directed activity), alogia (poverty of speech) and blunted affect (emotional flattening). While antipsychotic medication has been reasonably successful in treating positive symptoms, negative symptoms and cognitive dysfunction remain more enduring aspects of the illness (Seidman et al., 1993).

Mood symptoms and cognitive impairment are two additional symptom clusters in schizophrenia (Flashman and Green, 2004). Mood symptoms include depression, anxiety, and general dysphoria that may be present, and possibly exist separate from the active phase symptoms.

Cognitive impairment constitutes a separate domain of the illness that is independent of clinical symptoms (Gold and Harvey, 1993; Harvey et al., 2004; Kasper and Resinger, 2003; Keefe et al., 1999) and is present early in the illness (Bilder et al., 2000; Saykin et al., 1994). Cognitive dysfunction in schizophrenia includes deficits in attention and vigilance, executive function, verbal memory and learning, language and fluency, visuospatial ability, fine motor skills, and motor speed (Bilder et al., 2000; Gold and Harvey, 1993; Green et al., 2004; Saykin et al., 1991). The persistence of cognitive dysfunction in schizophrenia is believed to be responsible for the significant functional disability associated with the illness (Green, 1996; Green et al., 2000). Executive cognitive deficits, in particular, are persistent features of the disorder that are resistant to treatment and predictive of poor rehabilitation (Krystal et al., 2000).

Neurobiology

Currently, research supports a "neurodevelopmental hypothesis" of schizophrenia. This hypothesis suggests that an early aberration of brain development (e.g. prenatal, perinatal, or postnatal) interacts with central nervous system (CNS) maturational processes, leading to the emergence of the disorder (Weinberger, 1987). In effect, abnormal genetic and environmental events adversely affect early brain development, and the resulting neuropathology, over the course of CNS development, causes expression of the symptoms of schizophrenia. Abnormalities of cortical development, specifically in the migration and organization of neurons, are believed to create dysfunctional prefrontal temporolimbic cortical connectivity (Weinberger and Lipska, 1995), explaining the structural and functional abnormalities described in the

illness. The process of neuron formation and migration begins around the second trimester of pregnancy and ends in young adulthood (Andreasen, 2000). Because the illness typically emerges in late adolescence, this is believed to be a critical time during which molecular mechanisms regulating brain maturation are triggered (Feinberg, 1982).

Alterations in neuronal density and decreases in neuronal size in the limbic, temporal, and frontal regions have been described (Arnold and Trojanowski, 1996). The absence of gliosis (i.e. scarring due to excess neuroglia at sites of CNS damage) further implicates the greater relevance of aberrant neurodevelopmental processes (e.g. neurite formation, synaptogenesis, neuronal pruning, apoptosis) than neurodegenerative processes (Roberts et al., 1987). Excessive synaptic pruning or apoptotic mechanisms during brain development may contribute to volumetric losses described in the illness (Frangou and Murray, 1996). Cellular abnormalities such as neuronal disarray suggest disruption of proliferation or migration at the gestational period (reviewed in Miyamoto et al., 2003).

Anatomical neuroimaging studies in schizophrenia, using computed tomography (CT) and magnetic resonance imaging (MRI) have demonstrated ventricular enlargement, reduced whole brain volume, and specific reductions in cortical volume in various brain regions including the limbic system, frontal cortex, temporal cortex, basal ganglia, thalamus, hippocampal complex and cerebellum (Shelton and Weinberger, 1986; Woods et al., 1996; Cannon, 1996). Functional imaging studies have also described abnormal blood flow or glucose metabolism in the frontal lobes, temporolimbic, and basal ganglia regions (Bachneff, 1991;

Buchsbaum, 1990). Cognitive disturbances in the illness have been shown to be related to structural brain abnormalities in that whole brain volume (WBV) is correlated with neuropsychological test performance, and ventricle-to-brain ratio (VBR) is associated with decreased abstraction (reviewed by Antonova et al., 2004).

At the neurotransmitter level, the earliest hypothesis for schizophrenia has been a hyperactivity of the dopamine (DA) system. This hypothesis attributes positive symptoms of the disorder to excessive stimulation of DA D_2 receptors in the striatum (Laruelle et al., 2003). Evidence to support this view arises from the efficacy of antipsychotic drugs that primarily block dopamine D_2 receptors in ameliorating positive symptoms, and the psychotomimetic effect of stimulants that increase dopamine activity, such as amphetamine and cocaine (Carlson, 1986; Stahl and Wets, 1988; Mesulam, 1990). The resistance of negative and cognitive symptoms to D_2 receptor antagonism has led to the reformulation of the DA hypothesis to include hypoactivity of prefrontal DA D_1 receptors (Davis et al., 1991; Laruelle et al., 2003).

More recently, focus has shifted from the DA system to glutamatergic system abnormalities in schizophrenia. Administering noncompetitive antagonists of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors, such as phencyclidine (PCP) and ketamine, to healthy control participants have been shown to trigger positive, negative, and cognitive symptoms of schizophrenia (Javitt and Zukin, 1991; Olney and Farber, 1995; Jentsch and Roth, 1999). Likewise, pharmacological studies using NMDA receptor antagonists with patients diagnosed with the illness have produced a clinical state indistinguishable from a relapse (Malhotra et al., 1997). Agents known to enhance NMDA receptor function, via the glycine modulatory site

for example, also ameliorate symptoms of the disorder (Meador-Woodruff and Healy, 2000).

Further appeal for the glutamatergic model of schizophrenia arises from how well it fits with both the neurodevelopmental hypothesis and the dopaminergic abnormality seen in the illness (Olney and Farber, 1995). For example, in animal studies, administration of PCP to young rats induces corticolimbic neuronal damage in the brain in an age dependent manner (i.e. beginning in puberty and continuing into adulthood), suggesting that rats have a similar onset of risk to NMDA receptor hypofunction induced neuronal injury to humans (Farber et al., 1995). These results also confirm that an early lesion suppressing NMDA receptor function can result in structural brain changes in corticolimbic regions further along in the developmental trajectory, a pattern similar to what is seen in schizophrenia (Olney and Farber, 1995). Further, acute administration of NMDA receptor antagonists to rodents markedly increases the release of dopamine and glutamate in the prefrontal cortex and in subcortical regions, but chronic treatment results in decreased dopamine turnover in the prefrontal cortex, mimicking the dopamine dissociation described in schizophrenia (Deutch et al., 1987).

Proton magnetic resonance spectroscopy (^{1}H-MRS), a relatively new neuroimaging modality, has allowed for the exciting possibility of examining abnormalities in the glutamatergic system *in vivo*. ^{1}H-MRS is a noninvasive neuroimaging technique that can measure the concentration of metabolites in a region of the brain in a prescribed volume-of-interest (VOI). A brief review of the principles

of nuclear magnetic resonance and the proton spectroscopy literature as it relates to schizophrenia will now be undertaken.

Chapter 2

Neuroimaging: MRS

Principles of NMR

Magnetic resonance imaging (MRI) is based upon a characteristic of atomic particles known as "spin" in which certain nuclei, having an odd number of protons or neutrons, behave like small spinning charged particles, creating a magnetic dipole moment (Gadian, 1982). The term NMR, or nuclear magnetic resonance, refers to the fact that magnetic dipoles of atomic nuclei resonate when exposed to an external magnetic field (Bloch et al., 1946; Purcell et al., 1946). In 1952, Bloch and Purcell shared a Nobel Prize in Physics for using this principle to construct the first NMR spectrometer (Macomber, 1998). The hydrogen nucleus, containing a single proton, has a strong magnetic dipole moment and is abundant in organic tissue (Mansfield and Morris, 1982), especially as a component of water which constitutes 80% of brain weight (Pagnoni and Berns, 2004). Thus, hydrogen provides the strongest signal of MRI nuclei (Mansfield and Morris, 1982) and is the basis of MRI technology.

In tissue, protons are normally oriented in random directions, but if an external magnetic field (B_0) is applied, the protons will align either parallel or antiparallel to the magnetic field (Gadian, 1982). In addition to the parallel/ antiparallel orientation of protons, the static magnetic field induces "precession" in the protons, which means the protons spin about the axis of the static magnetic field similar to the way a spinning top "precesses" about the vertical gravitational force; the magnetic moment of a nucleus in an external magnetic field precesses with a characteristic angular

frequency known as the Larmor frequency (Gadian, 1982; Mansfield and Morris, 1982). The parallel orientation of protons in an external magnetic field requires a lower energy state than its antiparallel counterpart, and a change can be induced between these two energy states by applying a radiofrequency (RF) pulse at a corresponding resonant frequency (Gadian, 1982). When another magnetic field (B_1), that is perpendicular to B_0, and is much weaker than the external magnetic field, is applied using electromagnetic radiation at the same Larmor frequency as the nuclear magnetic moments, this RF pulse excites protons into a higher energy state transiently orienting their spins away from the applied magnetic field (Mansfield and Morris, 1982). Thus, when the RF pulse is removed, the protons return to their equilibrium state, releasing the radiofrequency energy they had absorbed which constitutes the actual magnetic resonance signal; this radiofrequency wave, or more accurately, the temporal signature of its decay as the excited spins relax, is then detected by a RF coil (Gadian, 1982), which in brain imaging studies takes the shape of a cylindrical "cage" surrounding the participant's head in the scanner. The signal, therefore, relies on both the particular sequence of RF excitation pulses used, as well as molecular qualities of the local tissue. The signal is then transformed by various mathematical processes into an image that can be viewed.

The relaxation of protons back to equilibrium is characterized by two time constants: T_1 recovery (longitudinal or spin-lattice relaxation) which is parallel to the magnetic field and T_2 decay (transverse or spin-spin relaxation) which is perpendicular to the magnetic field (described in Mansfield and Morris, 1982). T_1 relaxation time is the rate at which protons realign with the static magnetic field. Variation in T_1

between different tissue types is the primary source of contrast in images. T_2 relaxation time is the rate at which individual magnetic moments begin to lose their phase coherence and return to a random arrangement; this provides images that are influenced by inhomogeneity of the magnetic field and local blood perfusion (Mansfield and Morris, 1982). Consequently, T_1-weighted images precisely define brain anatomy and are used in neuroanatomical studies whereas T_2-weighted images highlight changes in vascular activity and are particularly suited for functional magnetic resonance imaging (fMRI) (Pagnoni and Berns, 2004).

The "echo" is the key signal used to create the MRI image. Spin echo imaging occurs when a 90° RF pulse follows a 180° RF pulse, which forms the echo (Mansfield and Morris, 1982). The repetition time of the echoes, TR, is the time between successive RF pulses, while the echo time, TE, is the time between the initial pulse and the center of the echo; TR and TE are the two parameters that are adjusted to obtain the desired contrast between tissues such that as TR is shortened, T_1 weighting increases, and as TE is lengthened, T_2 weighting increases (Mansfield and Morris, 1982).

Magnetic Resonance Spectroscopy (MRS)

MRS, magnetic resonance spectroscopy, operates on the same basic principles of NMR as seen in MRI. Rather than creating anatomical images, however, MRS creates a "frequency signal intensity" spectrum of peaks that reveals the biochemical composition of a particular region, with the earliest studies of biological systems dating back to the 1950's (Odeblad and Lindstrom, 1955) and 1970's (Mansfield and Maudsley, 1977). Proton MRS (^{1}H-MRS) is based on signal acquisition from

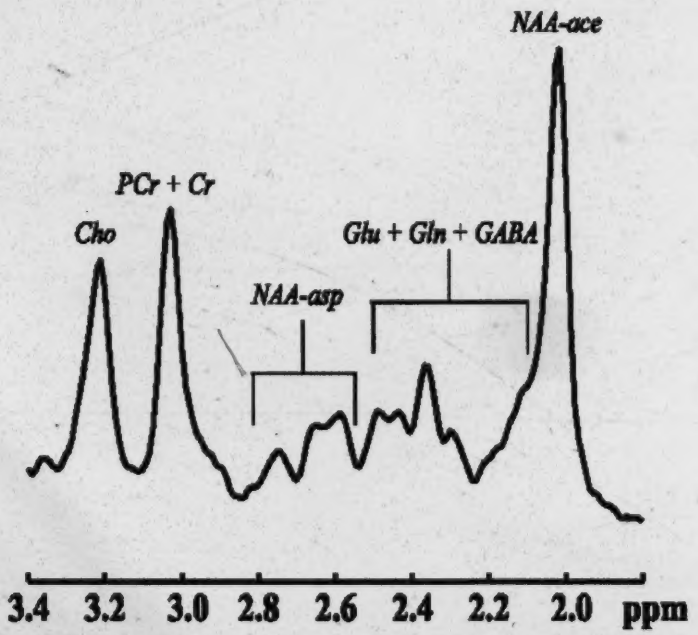
excitation of hydrogen atoms and relies on two main techniques: single-voxel ^{1}H-MRS and proton magnetic resonance spectroscopic imaging (^{1}H-MRSI) (Bertolino and Weinberger, 1999). The first technique examines signals from a localized, three-dimensional area, measured in "volume pixels", more commonly known as voxels, in which electromagnetic pulses with different frequencies for each of the three orientations of space are used to define the three dimensional volume of interest (VOI) (Mansfield and Morris, 1982). This allows for the creation of a spectrum from a single localized region. Conducting spectroscopy at higher field strengths allows for the selection of smaller voxel sizes, and greater spatial resolution as the signal-to-noise ratio is increased (Stanley et al., 2000). With ^{1}H-MRSI, one or more slices of tissue can be excited and signals from hundreds of smaller brain voxels are used to quantify neurochemicals from several brain regions simultaneously (Bertolino and Weinberger, 1999). Single-voxel techniques lack the precise anatomical characterization of ^{1}H-MRSI, making it difficult to differentiate between grey and white matter (Stanley et al., 2000). This is important in the context that grey matter and white matter are chemically distinct, in addition to being present in different concentrations (Keshavan et al., 2000). The single-voxel technique, however, allows for better quantification of metabolites when compared to ^{1}H-MRSI (Bertolino and Weinberger, 1999). In the literature, metabolites are either reported in absolute concentrations, or as ratios over choline, creatine, the signal of water, or noise (Bertolino and Weinberger, 1999). Since water is the most abundant molecule containing ^{1}H found in the brain, the signal of water is usually suppressed before acquisition of signals from other molecules (Bertolino and Weinberger, 1999).

The way in which MRS is able to recognize different molecules even though it is exciting only one nucleus (that of ^{1}H for example) is through a process called "chemical shift" (Gadian, 1982). Chemical shift refers to how clouds of electrons surrounding the nucleus create their own small local magnetic field variations that "shield" the nucleus, forcing it to resonate at a slightly lower frequency; nuclei in different chemical environments will create signals of different frequencies (Gadian, 1982). Therefore, even if molecules contain the same element (e.g. ^{1}H), nuclei of different molecules will possess different shielding based on local field variations due to the bond configuration of given molecules, allowing them to be recognized as distinct (Mansfield and Morris, 1982). To detect minute changes in "chemical shift", a very homogenous magnetic field is required and a process known as "shimming" is used to achieve this (Mansfield and Morris, 1982).

A process called Fourier transformation converts the digital version of the MRS signal from a function of time to a function of frequency, in which the frequency domain spectrum can be plotted (Macomber, 1998). Each metabolite has a unique position along the frequency axis measured in parts per million (ppm), and the area under the peak is proportional to the concentration of that particular metabolite (Gadian, 1982). In vivo levels of metabolites most often examined in ^{1}H-MRS studies include glutamate (Glu), glutamine (Gln), N-acetylaspartate (NAA), creatine (Cre), inositol (Ino), and choline (Cho).

A ^{1}H spectrum includes singlet resonances from NAA, phosphocreatine plus creatine (PCr+Cr) and trimethylamines (TMA's) at 2.01, 3.02, and 3.22 ppm respectively (Stanley et al., 2000). A Cho peak is found at 3.2 ppm (Bertolino and

Weinberger, 1999). Glu, Gln, and GABA resonate together at 2.1, 2.29, and in a region from 3.72 to 3.82 ppm, creating the combined Glx multiplet at lower field strengths (Bertolino and Weinberger, 1999). At higher field strengths, different peaks do not overlap quite so much as the chemical shift dispersion is greater; thus Glu and Gln are more accurately resolved with stronger magnets (Stanley, 2002). Since the majority of studies in schizophrenia have concentrated on NAA, Cho, and Glx, these three compounds will be examined further in a later section. Figure 1 shows a typical spectrum derived from a ^{1}H-MRS scan.



STEAM localised spectrum was acquired with TR = 3 s, TE = 20 ms, TM = 30 ms, number of averages = 256. Cho - choline, PCr - phosphocreatine, Cr - creatine, NAA- asp - aspartate resonances of N-acetylaspartate, Glu - glutamate, Gln - glutamine, GABA - γ-aminobutyric acid, NAA-ace - acetyl resonance of N-acetylaspartate

Figure 1. Representative spectra from ^{1}H-MRS scan of the medial frontal cortex showing major metabolite peaks.

In addition to hydrogen (^{1}H), different nuclei can be detected by MRS including carbon (^{13}C), fluorine (^{19}F), sodium (^{23}Na), phosphorus (^{31}P), and potassium

(^{39}K) (Mansfield and Morris, 1982), with ^{31}P and ^{1}H having the most widespread use in schizophrenia.

^{1}H-MRS Studies of Schizophrenia

Proton MRS provides information about neuronal metabolism (Gadian, 1982). A relatively consistent finding in schizophrenia is a decrease in NAA in different brain regions. NAA is often used a marker of neuronal mass and integrity as it is considered to be mainly intraneuronal (Urenjak et al., 1993). However, data showing that NAA decreases are possibly reversible (De Stefano et al., 1995), and are also present in oligodendrocytes (Bhakoo and Pierce, 2000) question this assumption. NAA synthesis takes place in the mitochondria and is ADP dependent. The synthetic reaction of NAA is a transamination catalyzed by L-aspartate-N-acetyl transferase that uses glutamate (as a source of aspartate) and either pyruvate or 3-hydroxybutyrate (as a source of Acetyl coenzyme A) as substrates (Stanley et al., 2000). Additionally, N-acetylaspartylglutamate (NAAG) can be cleaved by N-acetyl-α-linked acidic dipeptidase (NAALADase) to form glutamate and NAA (Tsai et al., 1995). Inhibition of mitochondrial energy metabolism reduces NAA concentrations, and this correlates highly with the relative reduction of adenosine triphosphate (ATP) and oxygen (O_2) consumption (Bates et al., 1996). Although the biological role of NAA is not clearly understood, it is believed to be involved in mediating metabolic efficiency, synthesizing myelin, and acting via the glutamatergic N-methyl-D-aspartate (NMDA) receptor to increase intracellular calcium (reviewed in Tsai and Coyle, 1995).

An early study showed a significant reduction in frontal, but not temporal NAA in a group of schizophrenia patients with mixed medication history (Buckley at

al., 1994). In a first episode sample, decreased NAA/Cre was reported in both frontal and temporal regions compared to a control sample (Cecil et al., 1999). Reports examining the temporal lobe specifically have found bilateral reductions of NAA/Cre in 16 stable, treated patients (Yurgelun-Todd et al., 1996), as wells as reduced NAA/ Cre + PCr in 13 first episode patients (Renshaw et al., 1995). Reduced NAA in the right hippocampus/ amygdala region of chronic patients has also been observed (Nasrallah et al., 1994). As well, decreased NAA/Cho and NAA/Cre in the medial temporal lobe of chronic patients has been found (Fukuzako et al., 1995). Lower NAA/ Cre have been reported in bilateral thalamus of schizophrenia patients (Deicken et al., 2000; Ende et al., 2003). Lower anterior cingulate NAA/ Cho was shown in patients after controlling for grey matter (GM) (Yamasue et al., 2002). Taken together, these studies suggest possible reduced neuronal integrity in the frontal and temporal regions in schizophrenia. This may lend support to the structural and functional abnormalities described in these regions in the illness, as well as bolster a neurodevelopmental origin for the illness. However, other studies have failed to find NAA reductions in frontal (Stanley et al., 1996) and temporal lobes (Buckley et al., 1994; Bartha et al., 1999), and the precise role of NAA in the body and the significance and specificity of NAA reductions is still unclear. Additionally, Lim and colleagues (1998) reported normal NAA values in the grey matter (GM) of patients, despite a volume reduction of 18%, in contrast to reduced NAA values in white matter (WM), highlighting again the need to address tissue segmentation. Because grey matter has greater neuronal density, NAA levels have been shown to be generally greater in grey matter, with NAA reductions seen in white matter (Lim et al., 1998).

Therefore, reports of NAA reductions in schizophrenia should include white matter/grey matter segmentation to determine where exactly the reductions are found.

Choline is a component of the phospholipid membrane structure; therefore, changes in the turnover of membrane phospholipids should be mirrored in changes to the Cho signal (Bertolino and Weinberger, 1999). Cell membranes consist of phospholipids arranged in two layers: the hydrophobic fatty acid layer facing into the membrane and the hydrophilic layer facing out to the extracellular fluid (ECF) or into the intracellular fluid (ICF) (Horrobin et al., 1994). Around 20 to 30 different fatty acids may attach to these two sites, and along with cholesterol esters, provide the structural framework for protein membrane components such as ion channels, receptors, and other components of second messenger systems. Thus, changes to membrane structure can affect all receptor sites associated with that membrane (Horrobin et al., 1994). This has implications for neurotransmitter action, and may provide an explanation for abnormalities in neurotransmitter function seen in schizophrenia (Laruelle et al., 2003).

Maier and Ron (1996) found age-related decreases in Cho levels of the hippocampus in their sample of 26 chronic schizophrenic patients which they suggest may reflect changes to membrane lipids and myelin without overall neuronal loss as NAA levels did not change over time in comparison to a control group. This may be due to altered lipid neurochemistry or an abnormal myelination process in schizophrenia (Maier and Ron, 1996; Horrobin et al., 1994). Although this research group quantified metabolite concentrations relative to the water signal, the entire schizophrenia sample was receiving neuroleptic treatment. The role of medication in

this finding is unclear as the type and dose of pharmacological treatments are not described. Thus, the age-related effect seen in this study is potentially confounded by possible changes produced by pharmacological treatment. In contrast, an increase in Cho/Cr in the left medial temporal lobe was reported by Fukuzako and colleagues (1995) in another group of 30 medicated schizophrenia patients. However, the authors state that there were no correlations between the metabolites they measured (Cho/Cr, NAA/Cho, NAA/Cr) and the daily dose of neuroleptic and anticholinergic medications, which were expressed respectively in chlorpromazine and benztropine equivalents. The actual medications that patients were taking are not described.

Other studies have demonstrated an effect of medication on Cho levels. For example, Théberge and colleagues (2003) found a higher level of Cho in the left thalamus of schizophrenia patients receiving atypical antipsychotic medication versus those receiving typical antipsychotics. Although this study used a small sample size to perform this analysis, the findings again emphasize the need to examine the effects of specific types of medication more closely.

Moore and colleagues (2002) did not find differences in Cho/Cr levels between patients and controls, but they found a decrease in Cho in the left temporal lobe in both groups that the authors suggest may be due to lateralized differences in grey and white matter in the voxel. Once again, differing grey and white matter mixtures provide conflicting results that limit the generalizations that can be made from the findings. These studies are ambiguous, but Cho involvement in phospholipid metabolism could suggest pathological changes to cell membrane structures,

indicative of cell damage leading to turnover of phospholipids which has been shown in demyelinating diseases (reviewed in Ross and Sachdev, 2004).

MRS studies often vary widely in terms of medication status, phase of illness, and methodology (i.e. voxel size and location, short versus long echo times, different pulse sequences, and choice of metabolite used to form the ratio) (Keshavan et al., 2000). Consequently, the conflicting results in the literature leave any conclusions drawn to be tentative ones at best. Various authors suggest ways to improve both reporting and methodology in spectroscopy. Firstly, because of the difficulty in using ratios that may confuse which neurochemical is contributing to the alterations seen, absolute concentrations of the neurochemicals should be used where possible (Stanley et al., 2000). For example, some authors have documented increases in Cho in patients with schizophrenia (Fukuzako et al., 1995; Théberge et al., 2003), therefore, using an increased Cho value in the denominator of the metabolite ratio could contribute to some of the decreased NAA levels reported in the literature. Some authors suggest measuring metabolites relative to the internal water concentration of the voxel, due to the larger 1000 fold increase in signal-to-noise ratio for the water signal (Bartha et al., 1999). Because the water signal is larger than the signal from other metabolites, it is more reliably quantified and is a more stable entity to compare to than the other metabolites. Secondly, segmentation data is important given the grey matter volume reductions seen in schizophrenia (Keshavan et al., 2000). If there is a disproportionate concentration of MRS metabolites in grey relative to white matter, any conclusions drawn regarding decreased levels of metabolites seen in patient groups is suspect unless grey and white matter mix in the area of interest is taken into

account. As well, since these grey matter reductions occur over the course of illness, distinctions need to be made between "state" and "trait" alterations seen in MRS metabolites (Keshavan et al., 2000).

Methods for localization, and the time of echo (TE) parameters also differ between studies. There are two main methods used for localization in spectroscopy research in schizophrenia: the stimulated echo acquisition mode (STEAM) and the point resolved spectroscopy (PRESS) pulse sequences (Stanley, 2002). Both sequences acquire the MR signal from the intersection of three orthogonal slices (sagittal, transverse, coronal) to localize either a single voxel or multiple voxels (Stanley 2002). The PRESS sequence uses one 90° slice selective pulse followed by two 180° slice selective pulses. The STEAM sequence uses three 90° slice selective pulses.

While some studies provide enough information to provide reliable reproduction of voxel placement, some inconsistencies remain in the literature. Detailed descriptions should be provided within the methodology section of publications to permit reliable reproduction of voxel placement for replication purposes. As well, settings for parameters such as TE, TR, and TM (mixing time) should be made explicit. There is a subtle balance that must be achieved in setting these parameters to optimize signals and reduce "noise" (Gadian, 1982).

While previous studies have mainly reported on NAA, reports on glutamate are relatively scarce. Since it is believed that NAA levels are highest in pyramidal glutamatergic neurons, the NAA decreases may be secondary to pathology in the

glutamate system (Urenjak et al., 1993). A review of glutamate, and its findings in schizophrenia, will now be explored.

Chapter 3

Glutamatergic Hypothesis of Schizophrenia

Glutamate

Glutamate is ubiquitous in the brain and presumed to be utilized by 40% of all synapses. It is the major excitatory amino acid neurotransmitter of the pyramidal cells connecting pathways of the cerebral cortex, thalamus, and limbic system (Tsai and Coyle, 2002). Glutamic acid (referring to glutamate's ionized form at physiological pH) is a non-essential amino acid (i.e. synthesized by the body and not required as part of the diet) (Feldman et al., 1997). Glu has other biological roles including detoxification of ammonia in the brain, as a building block in the synthesis of proteins and peptides, and as a precursor for the inhibitory neurotransmitter GABA. Excess Glu is excitotoxic and is known to cause neuronal damage and death, primarily by elevating cellular Ca^{2+} and exciting cells to death (Ankarcrona et al., 1995). This mechanism is seen in diverse pathologies such as ischemia, hypoxia, epilepsy, and Alzheimer's disease.

In vitro studies have found that glutamate concentrations in the frontal cortex are approximately 9.0 mmol kg wet weight, 80% of which is found in glutamatergic neurons, and the remainder in glial cells (Erecinska and Silver, 1990). These two compartments comprise the metabolic and neurotransmitter pools respectively. Extracellular glutamate is maintained at 2-3 μM by sodium dependent high-affinity uptake systems, both in nerve terminals, and also in the glia (Yudkoff et al., 1993).

Synthesis and metabolism

Glutamate is synthesized in the brain from two main sources: (1) glucose via the Kreb's cycle and transamination of α-ketoglutarate (α-KG), and (2) from glutamine (Cooper et al., 2003). The first instance involves the breakdown of glucose to pyruvate, conversion of pyruvate to acetyl coenzyme A (acetyl CoA), and the entry of acetyl CoA into the citric acid (Kreb's) cycle (Feldman et al., 1997). α-KG can then be converted to glutamate by means of transamination. In this process, enzymes called aminotransferases can transfer an amino group from a donor amino acid to an acceptor molecule (Feldman et al., 1997). If the acceptor molecule is α-KG, then a glutamate molecule is formed (Figure 2a). In the second instance, glutamine is converted to glutamate by the action of the enzyme glutaminase (Figure 2b). Glutaminase is a mitochondrial enzyme that requires ATP for its activity.

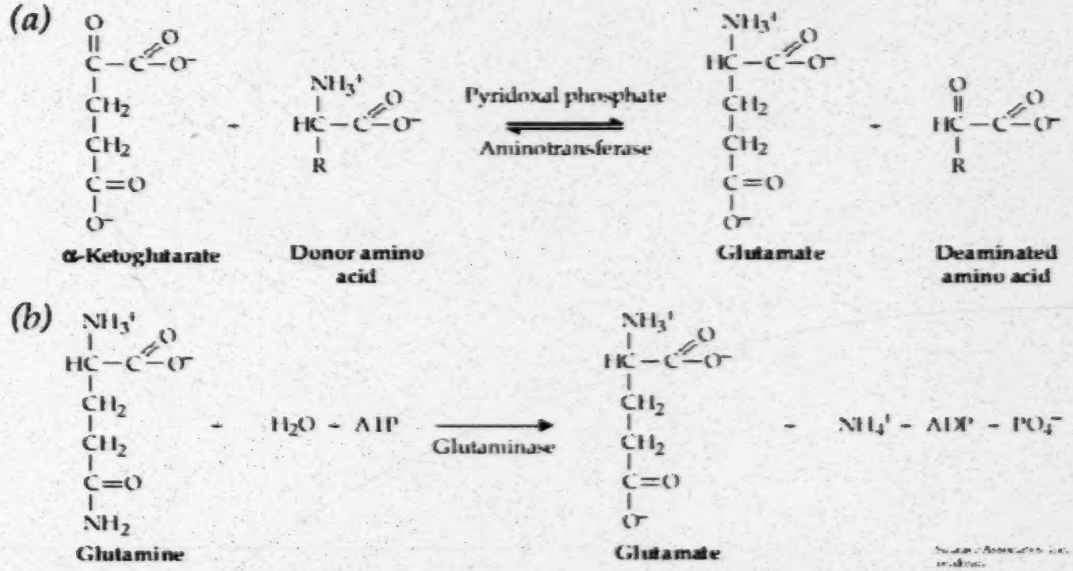


Figure 2. Glutamate synthesis by (a) transamination of α-ketoglutarate and (b) via glutamine (Source: Feldman et al., 1997, Fig. 10-3). Reprinted with permission.

Glial glutamate can likewise be converted back to glutamine via the action of glutamine synthetase (GS) (Feldman et al., 1997). Glutamate is released from the presynaptic terminal into the synaptic cleft. Excess glutamate is taken up by surrounding glia (astrocytes) and converted to glutamine via activity of GS, and partly to α-KG and ammonia via the action of glutamate dehydrogenase (GDH) (Burbaeva et al., 2003). Glutamine is released, taken up by the presynaptic neuron, and converted back into glutamate by mitochondrial glutaminase. Thus the synthesis of the neurotransmitter pool of glutamate is primarily derived from this glutamate - glutamine cycling. Glutamine is localized predominantly in glial cells (Erecinska and Silver, 1990).

Storage and Release

Glutamate is stored in synaptic vesicles. Once the nerve cell is depolarized, it is released into the synaptic cleft in a calcium-dependent exocytotic process (Cooper et al., 2003). Glutamate action is terminated by a high affinity reuptake process by glutamate transporters (GTs). There are two main types of plasma membrane GTs: those on the presynaptic nerve terminal (GTn) and those found in glial cells (GTg) (Feldman et al., 1997). GTs are found predominantly in axon terminals, have distinct cellular distributions and pharmacological properties, and have been shown to display high specificity for glutamate (Cooper et al., 2003). These excitatory amino acid transporters (EAATs) have been designated EAAT1 through to EAAT5 and are found in both glial cells and neurons (Nestler et al., 2001). These two types of cells have a comparable plasma membrane glutamate uptake carrier that terminates postsynaptic action and maintains manageable extracellular glutamate concentrations (Cooper et al.,

2003). By limiting concentrations of glutamate, extracellular GTs prevent excessive stimulation of glutamate receptors (Nestler et al., 2001).

Rapid removal of glutamate from the synapse is necessary in order to end the excitatory signal. If this high-affinity reuptake process does not occur, extracellular levels of glutamate can induce excitotoxic damage (Holden, 2003). This is thought to be a major process by which neuronal damage is incurred in neurodegenerative disorders and cerebral ischemia (Feldman et al., 1997).

Receptors

Five different types of glutamate receptors have been identified, and these fall into two major classes: ionotropic and metabotropic receptors. The specific receptors are named for the agonists acting at those sites. Ionotropic receptors belong to the ligand-gated channel receptor family and include NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid), and kainic acid receptors (Feldman et al., 1997). These receptors are characterized by their depolarizing excitatory action and by the blockade effect of these agonists by selective antagonists. All three ionotropic receptor families are mediated by the opening of cation channels permeable to Na^+ and, in a subtype-specific fashion, to Ca^{2+}, which depolarizes or "excites" the neuron (Goff and Coyle, 2001). These ligand-gated channels operate with a short latency and are particularly adapted for rapid signaling in the nervous system (Feldman et al., 1997).

Metabotropic glutamate receptors are linked by G proteins to cytoplasmic enzymes and affect intracellular metabolic processes (Cooper et al., 2003). Eight metabotropic glutamate receptors, encoded by a family of eight genes, have been

identified and are designated mGluR1 through to mGluR8 (Goff and Coyle, 2001). When a G protein molecule couples with an agonist-stimulated receptor, the G protein stimulates membrane effectors to mediate a cellular response, such as activating particular enzymes (protein kinases) that catalyze the phosphorylation of substrate proteins (Feldman et al., 1997). For example, these receptors act via phospholipase C or by inhibiting adenyl cyclase to modulate glutamatergic transmission in more complex ways (Tsai and Coyle, 2002). Consequently, metabotropic receptors are slower acting but are capable of more subtle modulatory action than ionotropic receptors.

Another glutamate receptor, which does not fall into the above two categories, is the AP4 (1,2-amino-4-phosphonobutyrate) receptor which functions as an inhibitory autoreceptor. Presynaptic autoreceptors exercise an inhibitory effect on transmitter release, thus providing a negative feedback function by down-regulating release and/ or synthesis to prevent overstimulation of the postsynaptic receptor site (Feldman et al., 1997).

NMDA Receptor

The N-methyl-D-aspartate (NMDA) receptor complex (Figure 3) possesses a glutamate recognition site to which receptor agonists and competitive antagonists bind, as well as other binding sites for glycine, polyamines (such as spermine and spermidine), PCP (and other related dissociative anaesthetics such as ketamine), Mg^{2+} (magnesium) and Zn^{2+} (zinc) (Cooper et al., 2003). At resting membrane potential, the NMDA channel is blocked by Mg^{2+} which is only removed upon depolarization due to the activity of AMPA and/or kainate receptors (Goff and Coyle, 2001). These non-

NMDA receptors open their ion channels once glutamate binds to them allowing the entry of positive ions into the cell. The influx of positive ions increases the cell voltage sufficiently enough to remove the voltage dependent Mg^{2+} block. Once the block is removed, the NMDA channel opens to permit an influx of Na^{+} and Ca^{2+} ions, and an efflux of K^{+} ions. (Goff and Coyle, 2001). This flow of ions results in depolarization of the plasma membrane and the generation of an electrical current, thereby "exciting" the neuron.

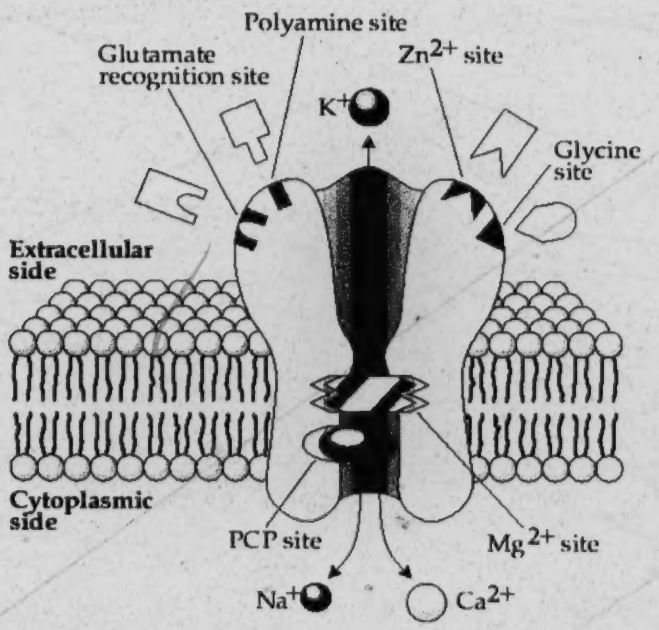


Figure 3. Representation of the NMDA receptor complex.
(Source: Feldman et al., 1997, Fig. 10-10). Reprinted with permission.

The strychnine-insensitive binding site for the co-agonist glycine plays an important modulatory role since it must be occupied in order for glutamate to open the ion channel (Goff and Coyle, 2001). Glycine has been a target for pharmacological interventions in schizophrenia and has shown some efficacy in treating negative symptoms (Heresco-Levy et al., 1999).

Hypoglutamatergic Hypothesis

The development of a hypoglutamatergic hypothesis of schizophrenia arises from converging lines of evidence spanning basic science and clinical work. These studies show widespread abnormalities in the glutamatergic system in schizophrenia ranging from dysfunction in glutamate binding to specific receptors and transporters, to problems in glutamate synthesis and metabolism (Burbaeva et al., 2003). These findings will now be reviewed in an attempt to delineate the role of glutamate in the etiology and pathophysiology of schizophrenia.

Kim and colleagues (1980) were among the first to report decreased concentrations of glutamate in the CSF of patients with schizophrenia, providing evidence for a possible hypoglutamatergic model of the illness. This finding was met with skepticism as a subsequent study examining glutamate CSF in schizophrenia failed to replicate these results (Perry, 1982). However, more recent studies have been able to provide evidence supporting a hypoglutamatergic model. Postmortem studies in schizophrenia have described abnormalities of the density and subunit composition of glutamate receptors in the prefrontal cortex, thalamus, and hippocampus (reviewed in Meador-Woodruff and Healy, 2000; Gao et al., 2000; Ibrahim et al., 2000). The NR1 subunit is important for regulating and expressing functional NMDA receptors; the NR1 subunit combined with one of four NR2 subunits constitute the NMDA receptor complex (Meador-Woodruff and Healy, 2000). Gao and colleagues (2000) found that levels of mRNA for NMDA receptor subunits NR1 and NR2B were different between individuals with schizophrenia and controls in postmortem hippocampal tissue. Likewise, lower levels of mRNA for NR1, NR2B, and NR2C

were seen in dorsomedial and central medial thalamic nuclei in patients with schizophrenia (Ibrahim et al., 2000).

Consistent with the previously mentioned clinical effects of NMDA antagonists such as PCP, results from animal models suggest NMDA hypofunction can likewise produce symptoms reminiscent of schizophrenia. Mohn and colleagues (1999) report on genetically altered mice expressing only 5% of normal levels of NR1 subunit who display the same behavioural abnormalities triggered by PCP. These behavioural abnormalities, such as increased motor activity and social deficits, are often used in animal models to represent schizophrenic positive and negative symptoms respectively. Interestingly, the behaviours improved with administration of antipsychotics, with clozapine showing a slight advantage over haloperidol (Mohn et al., 1999). Although both these antipsychotics have dopamine receptor blockade (D_2 for haloperidol, and D_2 and D_1 receptors for clozapine), the ability of the atypical antipsychotic (clozapine) to ameliorate the more enduring social deficits may be due to its ability to affect receptors for other neurotransmitters, such as 5-hydroxytryptamine (5-HT, serotonin), which may in turn enhance glutamate release (Mohn et al., 1999). This suggests that an underlying dysfunction in the glutamate system can be treated by antipsychotics acting on dopaminergic and serotonergic systems, which provide additional evidence of how these neurotransmitters mutually affect each other (Mohn et al., 1999).

Abnormalities have also been identified in terms of glutamate metabolism. Burbaeva and colleagues (2003) reported decreased immunoreactivity levels of glutamine synthetase (GS) and increased glutamate dehydrogenase (GDH) (involved

in glutamate catabolism to α-ketoglutarate) enzymatic activity in postmortem brain tissue of the prefrontal cortex (PFC) of patients with schizophrenia compared to controls. This provides evidence of metabolic dysfunction of the glutamate system in the PFC, which suggests one mechanism of explaining the characteristic frontal lobe deficits seen in this illness. These data support results found by Gluck and colleagues (2002) which showed that the activity of glutamic acid decarboxylase (GAD) (converts glutamate to GABA) and phosphate-activated glutaminase (PAG) (converts glutamine to glutamate, possibly representing a compensatory response to normalize glutamate levels) was significantly greater in postmortem dorsolateral prefrontal cortex of individuals with schizophrenia than in a comparison group.

Although this does not provide direct evidence of hypoglutamatergia, a dysfunction in the activity of these enzymes lends support to glutamate dysregulation. Burbaeva and colleagues (2003) speculated on where in the process the primary dysfunction occurs. For example, if increased GDH occurs first, decreased GS levels may occur as a compensatory mechanism to balance glutamate levels. Conversely, if decreased GS occurs first to raise glutamate levels, then genes encoding GDH might be induced to increase GDH levels (Burbaeva et al., 2003). In either case, the levels were significantly different from controls, implying dysfunction of the glutamate system.

Likewise, elevated levels of N-acetylaspartylglutamate (NAAG) (an NMDA receptor antagonist) in the hippocampus, and decreased glutamate levels and activity of N-acetyl-α-linked acidic dipeptidase (NAALADase) (which cleaves NAAG to glutamate and N-acetylaspartate) were found in prefrontal and hippocampal

postmortem brain tissue of schizophrenic patients (Tsai et al., 1995). This suggests decreased glutamatergic activity in these areas. NAAG is catabolized to N-acetylaspartate (NAA) and glutamate by the action of glutamate carboxypeptidase II (GCP II), and the activities of both NAAG and GCP II have been shown to be altered in a variety of neuropsychiatric disorders, including schizophrenia (Coyle, 1997). These results are again especially interesting considering these alterations occur in brain regions that have been implicated in the pathophysiology of schizophrenia.

Studies investigating glycine, a co-agonist at NMDA receptors, has likewise produced interesting results. Treatment with oral glycine has been shown to improve the more enduring negative symptoms of the illness (Heresco-Levy et al., 1999; reviewed in Goff and Coyle, 2001). Decreases in plasma levels of glycine, consistent with a hypoglutamatergic hypothesis of schizophrenia, were found in a study comparing 144 patients with schizophrenia to a group of control subjects and to a group of patients with depression (Sumiyoshi et al., 2004). The number of glycine binding sites has also been found to be elevated in schizophrenic brains (Ishimuru et al., 1992), which the authors postulated to be a result of compensatory receptor sensitization (i.e. an attempt to attenuate the consequences of NMDA hypofunction).

Glutamate and other neurotransmitters

Any model focusing on the effects of a single transmitter will be an oversimplification. It is known that neurotransmitters mutually influence the activity of one another (Olney and Farber, 1995). The prevailing dopamine hypothesis of schizophrenia, therefore, need not be wholly discarded in order to accept the crucial role played by glutamate. The dopamine (DA) hypothesis has been formulated to

include evidence supporting subcortical striatal D_2 hyperactivity (accounting for positive symptoms), and prefrontal D_1 hypoactivity (accounting for negative and cognitive symptoms) (reviewed by Tsai and Coyle, 2002; Laruelle et al., 2003). More specifically, these authors' reviews support the notion of cortical DA deficit contributing to subcortial DA hyperactivity as the former's role is to provide an inhibitory effect on subcortical processes. The current challenge has been elucidating the role of glutamate against this background of DA dysfunction. Cortical and subcortical structures connect functionally through glutamate action, so dysfunctional glutamate neurotransmission could lead to the DA dissociation described above (Meador-Woodruff and Healy, 2000). As well, it has been observed that glutamate and DA display reciprocal actions such that DA receptor blockade by antipsychotics may act to balance a hypoglutamatergic state (Carlsson et al., 2004).

Laruelle and colleagues (2003) reviewed the attempts of various researchers to explain how DA and glutamate interact in the brain. Cortical glutamatergic afferents and DA projections converge on GABAergic striatal neurons where DA displays modulatory effects on glutamate transmission (specifically, D_2 receptor stimulation inhibits NMDA-mediated glutamate transmission and D_1 receptor stimulation facilitates glutamate transmission) (Laruelle et al., 2003).

> By blocking D_2 receptors (and, at least acutely, stimulating D_1 receptors by augmenting DA release), antipsychotic drugs restore GLU transmission in the striatum, the ability of the striatum to receive and process cortical information, and the plasticity required for the shaping of cognitive processes by experience (Laruelle et al., 2003, p. 151)

In the prefrontal cortex, the authors summarize findings showing that D_1 receptors are located on pyramidal cells, and D_1, D_2, and D_4 receptors are localized on GABA

interneurons, allowing DA to modulate pyramidal cell excitability by modulating GABAergic interneurons (i.e. facilitating GABA inhibition of pyramidal cells) (Laruelle et al., 2003). Grunze and colleagues, as cited in Tsai and Coyle (2002), found that GABAergic interneurons were ten times more sensitive to NMDA receptor inhibition (using subanaesthetic doses of dissociative anaesthetics) than pyramidal neurons, which would result in loss of GABAergic inhibition, and subsequent impairment in cognitive processing. A glutamatergic deficiency would impair this neurotransmitter's usual influence on GABAergic inhibition (Deutsch et al., 2001). If NMDA receptors drive inhibitory GABAergic synapses on excitatory neurons, then NMDA receptor hypofunction would decrease GABA's inhibitory control over excitatory neurons (Olney and Farber, 1995). This uncontrolled excitation of cortical neurons could then possibly lead to excitotoxicity and subsequent neuronal damage over time (Holden, 2003). The lack of glutamatergic excitation of GABA inhibitory neurons leads to the dysregulation of multiple pathways, consequently disrupting the underlying function of these neurons. Byne and colleagues (1999) suggest that there may be heterogeneity in glutamate dysfunction such that a hypoglutamatergic state may exist at some synapses, while a hyperglutamatergic state may exist at others, mimicking the revised formulation of the DA hypothesis.

Glutamate and MRS

One of the first studies examining the glutamate system *in vivo* in first episode patients provides compelling clinical evidence to support the hypoglutamatergic hypothesis, thus far only supported by post-mortem and animal work. Bartha and colleagues (1997) used ^{1}H-MRS to examine levels of glutamate (Glu) and glutamine

(Gln) in the left medial prefrontal cortex (mPFC) of 14 first episode patients compared to 10 healthy controls. The authors found a significant increase in Gln in schizophrenic patients, indicating dysfunction in the conversion of Gln to Glu (Bartha et al., 1997). Higher levels of Gln would suggest lower glutamatergic activity as there would be decreased Glu available for signal transduction (Bartha et al., 1997). The authors did not find a difference in Glu, however, but they postulate that if the primary pathology lies in a dysfunction in the conversion of Gln to Glu, the overall cellular Glu would be unchanged (Bartha et al., 1997).

Another early study, investigating first episode and chronic patients, found no differences in drug-naïve patients, but increases in Gln levels in the left dorsolateral prefrontal cortex (DLPFC) of acutely medicated and chronic patients (Stanley et al., 1996). These studies, however, used a 1.5 Tesla (T) magnet for scanning and some authors have argued that the resonances for Glu and Gln cannot be reliably resolved at this field strength (Keshavan et al., 2000; Stanley, 2002). Ohrmann and colleagues (2005b), reporting on a combined Glx (Glu, Gln, GABA) peak at 1.5T, found decreased Glx levels in the DLPFC in a chronic sample of 21 patients compared to 18 first episode patients and 21 matched controls. This reduction was not correlated with duration of illness or medication.

Théberge and colleagues (2002), using a higher field strength 4.0T magnet, found an increase in Gln in the left anterior cingulate and thalamus of never-treated, first episode patients compared with healthy volunteers. Taken together, these results could suggest higher than normal glutamatergic activity as Glu is derived from Gln (Rothmann et al., 1999), or conversely, it may indicate hypoglutamatergia if there is

dysfunction in the conversion of Gln to Glu as suggested by the authors (Bartha et al., 1997; Théberge et al., 2002). This same research group examined the left anterior cingulate of a chronic sample of 21 patients with schizophrenia and found significantly lower levels of Glu and Gln (Théberge et al., 2003). However, Gln levels were found to be higher than normal volunteers in the left thalamus. The authors interpreted the decreased levels of Glu and Gln to possibly be related to neurodegeneration from long exposure to illness or the effects of chronic medication which could lead to dysregulation in the thalamus (Théberge et al., 2003). This interpretation is challenged by the Ohrmann (2005b) research group who failed to find such a relationship. In direct contrast to these findings, a recent study of 21 patients with chronic schizophrenia found that absolute concentrations of Glu were higher in the prefrontal cortex and hippocampus (van Elst et al., 2005). Some authors suggest that since the *in vivo* concentration of Glu is greater than Gln (Pouwels and Frahm, 1998) and reflects both the metabolic and transmitter pools of Glu, consequently, ^{1}H-MRS studies should use Gln values as it may be a more sensitive marker of glutamatergic neurotransmission, since Gln is directly involved in the synthesis of the transmitter pool of Glu (Stanley et al., 2000).

The relationship between PFC MRS and cognitive functioning is still a relatively new area requiring further investigation. Studies in this area would help elucidate the neurochemical underpinnings of cognitive processes. In the following chapter, a brief review of executive functioning, and its structural and neurochemical correlates will be examined further.

Chapter 4

Executive Dysfunction in Schizophrenia

Definition of Executive Function

To function efficiently in daily life, one has to monitor the external world, attend to relevant information within it, input and retrieve required information from storage, manipulate, integrate, and deliver task relevant information and behaviour, while simultaneously suppressing unwanted information and behaviour (Funahashi, 2001; Antonova et al., 2004). These numerous tasks require an integrative control network. Executive function encompasses identifying and categorizing task relevant information, developing strategies or acquiring rules necessary for task performance, and inhibiting redundant or ineffective responses (Lezak, 1995). Consequently, executive function is a general term referring to the complex cognitive integration and coordination of several subprocesses to achieve a specific goal; these sub-component processes involved in the executive control network include selective attention, short term storage of information, working memory, response inhibition to irrelevant information, response initiation to relevant information, and the self-monitoring of performance (Funahashi, 2001). Simply put, executive function involves the ability to plan, perform, and monitor one's own behaviour (Minassian et al., 2003).

Various neuropsychological tests (NPTs) are available commercially to assess different aspects of executive function (described in Lezak, 1995). The most common tests reported in the literature include the Wisconsin Card Sorting Test (WCST) (Heaton, 1981), Trailmaking Test (Russel et al., 1970), Halstead Category Test (Gregory et al., 1979), Stroop Color-Word Test (Golden, 1976), verbal/ design fluency

(Lezak, 1995; Jones-Gotman and Milner, 1977) and Wechsler Adult Intelligence Scale – Third Edition (WAIS-III) Digit Symbol subtest (Wechsler, 1997). These NPT's examine set shifting, mental sequencing, conceptual reasoning, response suppression, information processing speed, and attention.

Numerous cognitive models have been postulated to describe how executive function is achieved. Wood and Grafman (2003) contrast some of these models in terms of whether they employ a processing or representational perspective in the prefrontal cortex (PFC). For example, Duncan's (2001) adaptive coping model suggests that working memory, attention, and cognitive control are possible due to the adaptable nature of PFC neurons in coding task-specific, context-dependent states that are flexible and capable of change - a processing approach. Likewise, Norman and Shallice's supervisory attentional system (SAS) is an attentional control model in which the SAS is able to prioritize actions, and inhibit behaviours that are not required at any given moment (described in Marczewski et al., 2001). The authors also offer their own structured event complex (SEC) framework which emphasizes a representational approach in the PFC which is capable of storing unique types of knowledge (Wood and Grafman, 2003).

These models allow a hypothesis driven approach to determining structure-function relationships, and provide an explanatory framework in which to situate research findings. For example, the cognitive control mechanism described above is believed to be subserved by the anterior cingulate cortex and is illustrated by tasks such as the Stroop Test (Carter et al., 1998; Peterson et al., 1999). Event-related fMRI has demonstrated activation of bilateral PFC during set-shifting in the WCST (Konishi

et al., 1998) and activation in the medial prefrontal cortex (mPFC) during response inhibition on a go/ no-go task (Konishi et al., 1999). Difficulties with inhibiting prepotent responses can be viewed as a failure of the supervisory attentional system to suppress an activated schema inappropriate to the current context (Chan et al., 2004). By determining which structures are activated during normal executive functioning in healthy individuals, patterns of deficit and abnormal activation can be examined in schizophrenia. The explanatory power of these models to describe possible mechanisms of executive dysfunction can also be explored.

Executive dysfunction in schizophrenia is well documented in terms of set shifting, conceptual reasoning/ abstraction, volition, response inhibition, and problem solving (Gold and Harvey, 1993; Cools et al., 2000; Seidman et al., 2002). Johnson-Selfridge and Zalewski's (2001) meta-analysis of executive function in schizophrenia reported deficits of one and a half standard deviations below healthy volunteers (Δ=-1.45), and almost half a standard deviation below other psychiatric groups (Δ=-0.40). Most studies use the WCST as their test of choice. The WCST requires subjects to sort a deck of cards based on specific sorting criteria (i.e. colour, shape, number) that is not disclosed during the test session. Individuals must use feedback to determine whether they are using the correct sorting criterion and shift cognitive set when appropriate. The WCST examines abstract reasoning, conceptual set shifting, and working memory. On the WCST, schizophrenia patients complete fewer categories and make more perseverative errors (Kolb and Whishaw, 1983; Weinberger et al., 1986; Beatty et al., 1994). An event related fMRI study attempting to dissociate the neural circuitry involved in alternating behaviour and shifting cognitive set found that

response shifting activated a dorsal neural circuit (DLPFC, anterior cingulate, intraparietal sulcus) while shifts in cognitive set were mediated by the ventrolateral PFC, anterior cingulate, and striatum (Shafritz et al., 2005). Dysfunction in shifting cognitive set and alternating behaviour in schizophrenia may be due to abnormal circuitry in these regions.

Neuroimaging studies using positron emission tomography (PET) have shown abnormal activation in patients while performing executive tasks. A PET study of medicated patients with schizophrenia performing the Stroop task revealed that patients make more errors when naming color-incongruent stimuli (Carter et al., 1997). Both patients and healthy controls showed a direct correlation between anterior cingulate and hippocampal blood flow and total number of incongruent trial errors, but patients abnormally activated the anterior cingulate (Carter et al., 1997). This study was replicated by Nordahl and colleagues (2001), using higher resolution PET-600 and a longer acting [F-18] fluordeoxyglucose tracer to measure metabolic activity in patients with paranoid schizophrenia. They found that anterior cingulate glucose metabolic rate was positively correlated with total incongruent trial errors (Nordahl et al., 2001). This lends some support to the theory of performance monitoring by the anterior cingulate cortex, which rather than monitoring errors, may be more involved in detecting high levels of response competition (Carter et al., 1998). However, one PET study examining the effect of graded memory tasks showed deteriorating DLPFC activity in patients with schizophrenia as task demands increased, suggesting that hypofrontality may be more related to performance level (Fletcher et al., 1998b).

Equivalent performance is an important consideration when evaluating functional imaging studies as it presents a potential confound.

Intuitively, the relevance of executive skills to everyday function is apparent. In fact, card sorting has been shown to predict community functioning in schizophrenia, but not social problem solving (Green, 1996). Additionally, executive deficits in schizophrenia specifically affect activities of daily living (ADL) such as choosing a menu, shopping, or preparing a meal (Semkovska et al., 2004). The structures and circuitry subserving these processes will now be examined.

Frontal Lobe Function and Connectivity

Executive skills require intact frontal lobe functioning (Stuss and Benson, 1984). However, connections from the frontal lobes to sensory, limbic, and subcortical regions suggest executive dysfunction can also occur as a result of damage to any of these areas (Johnson-Selfridge and Zalewski, 2001). Thus, any executive deficit observed may be the result of a lesion somewhere else in the circuit (Anderson et al., 1991). Likewise, although patients may exhibit frontal lobe deficits on specific executive tests, their frontal lobes may be neurologically intact (Elliott, 2003). And conversely, test performance may fall within normal limits despite gross impairments in real-world behaviour and damage to frontal lobe structures. Lack of specificity, predictive validity, and ecological validity are particular criticisms aimed at tests of executive function.

Measuring frontal lobe function has been extremely difficult given the relative complexity of the area, and its rich networks with various other brain regions. The entire cortex in front of the central sulcus is considered frontal cortex; moving

anteriorly from the primary motor cortex, premotor cortex, and the supplementary motor area is the prefrontal cortex (PFC) which constitutes about 25% of the entire cortex (Diamond et al., 2002). The cytoarchictecture of the PFC is complex and disagreement exists on the boundaries of specific fields, and divisions of functions within the PFC (Brett et al., 2002). Generally, the PFC can be divided into ventromedial and dorsolateral regions, each with connections to posterior and subcortical brain regions. The ventromedial PFC (vPFC) has reciprocal connections to the amygdala, hippocampus, and sensory association areas, as well as to the DLPFC; the DLPFC has reciprocal connections to the basal ganglia, premotor cortex, supplementary motor area, cingulate cortex, parietal cortex, and association areas (Wood and Grafman, 2003). The DLPFC extends over superior and middle frontal gyri (Broadmann's areas [BA] 9, 46, 8 [posterior portion], and 10 [anterior portion]), while the vPFC lies on the interior frontal gyrus and covers BA 44, 45, 47/12 (Diamond et al., 2002).

Frontal-subcortical circuits can be grouped together based on anatomy and function. As many as five have been listed including the motor circuit, oculomotor circuit, orbitofrontal circuit, anterior cingulate circuit, and the dorsolateral prefrontal circuit (Saint-Cyr et al., 2002). The orbitofrontal cortex (OFC) includes the cingulate gyrus, parahippocampal cortex, temporal pole, and the insula (Mesulam, 2002). The orbitofrontal circuit consists of two subcircuits originating in BA 10 and 11. This circuit sends fibers to the ventral striatum and to the dorsal part of the nucleus accumbens, while the lateral orbitofrontal circuit sends projections to the ventromedial caudate (Saint-Cyr et al., 2002). The anterior cingulate cortex forms a medial-frontal-

subcortical circuit with the nucleus accumbens (NA) and the amygdala (Sbordone, 2000), and mediates motivated behaviour (Saint-Cyr et al., 2002). These structures also receive information from the ventral tegmental area (VTA), substantia nigra (SN), dorsal raphé nucleus (DRN), and the mediodorsal nucleus of the thalamus (Sbordone, 2000). The dorsolateral circuit originates in BA 9 and 46, has contributions from the parietal and temporal lobe association areas, and connects to the dorsolateral head of the caudate nucleus and the lateral aspect of the globus pallidus (Saint-Cyr et al., 2002). It has a direct pathway to the lateral substantia nigra, pars reticulata, and an indirect pathway through the ventromedial subthalamic nucleus. This circuit subserves most of the integrative cognitive control seen in executive function.

Functionally, the PFC is not required for encoding modality specific representations, but in regulating the selection, timing, monitoring, and interpretation of behavior (Mesulam, 2002). It provides the crucial basis for executive functioning through "top-down" modulation of various networks in the brain through other cortical and subcortical areas. Damage to the DLPFC has been reported to produce a neurobehavioural syndrome characterized by an inability to maintain set, dissociation between verbal and motor behaviour, deficits in complex or programmed motor activities, concrete thinking, poor mental control, and stimulus bound behaviour (Sbordone, 2000). Evidence from numerous clinical studies implicates abnormal frontal circuitry in schizophrenia (reviewed in Bunney and Bunney, 2000), however, the neurochemical basis of executive functioning is poorly understood.

Neurochemical Basis of Executive Functioning

The neurochemical bases of executive functions are not well delineated. Although a considerable body of research with animals provides clues relating to neurochemistry, generalization to more complex human behaviour has been limited. Regardless, *in vivo* microdialysis and voltammetry techniques have been used in animals to study the relationship between neurotransmitter release (proxy measure of changes in neuronal activity) and cognitive processes (Bruno et al., 1999). The majority of studies have focused on dopaminergic and cholinergic systems, although other transmitter systems have been studied.

Dopamine (DA) has been shown to affect PFC working memory function in monkeys (Brozoski et al., 1979; Sawaguchi and Goldman-Rakic, 1991), and research in animals has confirmed the importance of D_1 receptors in the regulation of PFC function (Arnsten and Robbins, 2002). DA receptor stimulation follows an "inverted U-shaped" dose response function in which DA depletion or excessive DA stimulation can impair working memory (Arnsten and Robbins, 2002). Increased DA release in the PFC is also one mechanism to explain the effects of stress on cognitive function (Arnsten and Goldman-Rakic, 1998). In psychiatric disorders, stress contributes to exacerbation of symptoms and relapse (Moghaddam, 2002).

Degeneration of the basal forebrain cholinergic system in disorders such as Alzheimer's disease and the corresponding intellectual decline implicates the cholinergic system with cognitive functioning (Arsten and Robbins, 2002). Intra-PFC infusions of scopolamine (an anticholinergic drug) to rats impair working memory ability on delayed matching and non-matching tasks (Broersen et al., 1995).

Acetylcholine's (ACh) action in the PFC may relate to its complex interactions with DA and Glu. Ach has been shown to enhance striatal DA release through nicotinic and muscarinic receptor activation on DA terminals, and NMDA receptor blockade decreases basal Ach release (reviewed in Saint-Cyr et al., 2002).

One meta-analysis of four behavioural tasks (Morris water maze, radial maze, passive avoidance, spontaneous alternation) used to examine learning and memory in the rat found that glutamate, GABA, dopamine, and acetylcholine had a greater impact (81-93%) on cognitive processes than serotonin and norepinephrine (48-55%) (Myhrer, 2003). Of these, glutamate ranked the highest overall. Long-term potentiation (LTP) is the suggested mechanism underlying the cellular basis of learning and memory, of which glutamate receptors play a key role; LTP is the strengthening of the connection between nerve cells resulting in a long term increase in the size of the post synaptic response, thereby increasing synaptic efficacy (Malenka and Nicoll, 1999). Because NMDA receptors require substantial neuronal depolarization in order to be recruited, via the co-activation of AMPA/ kainate receptors, they function as "coincidence detectors" which forms the basis of use-dependent synaptic plasticity (Goff and Coyle, 2001).

Animal studies have also suggested a role of the NMDA receptor complex in executive skills including rule acquisition/ implementation, set-shifting ability, and response inhibition (Murphy et al., 2005; Stefani et al., 2003). Specifically, NMDA and AMPA receptor blockade in the mPFC of rats impairs the ability to modify existing knowledge (between brightness and texture cues in a maze based task) and inhibit inappropriate responses (Stefani et al., 2003). Bilateral lesions in mPFC by the

injection of ibotenic acid selectively impairs shifting of attentional set, suggesting mPFC in rodents serve a similar function to primate lateral PFC (Birrell and Brown, 2000). Deficits in attentional accuracy were observed following intra-PFC infusion of a competitive NMDA receptor antagonist (Murphy et al., 2005), further suggesting glutamate's role in aspects of executive functioning.

Attempts to link aspects of human executive dysfunction with specific neurochemical abnormalities are rare, but would provide important clues to the underlying disturbance resulting in this disabling aspect of the illness. A brief review of the existing literature examining the relationship between MRS and cognition will first be explored.

Existing MRS and Cognition Literature

MRS allows for the detection of in vivo brain chemicals that may be relevant to cognitive processes. Studies in healthy volunteers reveal that NAA is associated with overall neuropsychological performance, especially with timed measures (Jung et al., 1999). Metabollically, greater NAA concentrations may assist with sustained attention on timed tasks, or NAA increases may be present in larger neurons suggesting greater conduction speed (Jung et al., 1999). Further, if healthy volunteers are stratified according to IQ, they differ significantly in total neuropsychological performance and in NAA levels (Jung et al., 2000). These studies in healthy individuals suggest that MRS is sensitive enough to differentiate variations in brain functioning (Ross and Sachdev, 2004).

Grachev and colleagues (2001) examined NAA levels in the right and left anterior cingulate cortex (ACC), DLPFC, OFC and thalamus, and cognitive

interference using the Stroop task in 15 healthy volunteers. NAA levels were reduced in the right ACC in high interference subjects in comparison to the low interference group. Cognitive interference was highly correlated with right ACC NAA. These findings again provide evidence of ACC involvement in cognitive interference, and a possible neural mechanism for the process. This study highlights the utility of using MRS to investigate cognitive control mechanisms.

MRS has also been used in schizophrenia to examine executive dysfunction in working memory and attentional processes. Braus and colleagues (2002) demonstrated an association between NAA in the anterior cingulate of patients with schizophrenia and perseveration errors on the WCST. Additionally, they observed increased levels of NAA were correlated with time on atypical medications and better performance. The authors hypothesize that atypical antipsychotics may restore the function (as measured by WCST errors) and viability (as measured by NAA) of anterior cingulate neurons in schizophrenia. As mentioned earlier, the AC has important functions within the executive control network and enhancing its function may improve executive function.

NAA levels in the DLPFC have also been shown to predict PET activation of working memory circuits during working memory tasks in two independent groups of schizophrenia patients (Bertolino et al., 2000). Similarly, NAA/ Cre in the DLPFC correlated with performance on the N-back memory working task in patients with schizophreniform disorder (Bertolino et al., 2003). These results provide support to neuronal pathology in the DLPFC contributing to working memory deficits seen early in illness onset. Taken together with the study by Braus and colleagues (2002), NAA

has been shown to be sensitive to aspects of executive function and to the effects of medication on cognitive processes.

Proton MRS investigations of glutamate and executive function in a first episode sample have not been examined. However, direct examination of NMDA receptor involvement in cognition has been shown using ketamine, a non-competitive NMDA glutamate receptor antagonist which can cause transient NMDA receptor hypofunction. Both direct and indirect pathways start with excitatory glutamatergic projections from the frontal cortex to the striatum (Saint-Cyr et al., 2002). The dramatic effects of PCP and ketamine reflect glutamatergic mediation of cortico-cortical, cortico-limbic, and cortico-subcortical communication (Cotman et al., 1995). The localization of NMDA receptors in cortical and limbic regions provides an explanation for the effect of NMDA receptor antagonism on various aspects of cognition, perception, and mood (Krystal et al., 1999b). Abnormal NMDA receptor-mediated glutamatergic neurotransmission has been implicated in the cognitive deficits seen in schizophrenia through ketamine impaired performance on tasks requiring frontal and hippocampal involvement such as the Wisconsin Card Sorting Test and Wechsler Memory Scales subtests of declarative verbal recall (Malhotra et al., 1997; Newcomer et al., 1999).

A recent MRS study by Rowland and colleagues (2005b), who administered subanaesthetic doses of ketamine to 10 healthy volunteers, found a significant increase in anterior cingulate glutamine. This change in glutamine was negatively correlated with Stroop performance, although the correlation did not reach statistical significance (Rowland et al., 2005b). This study is important for a couple of reasons. Firstly, it

provides evidence that direct NMDA receptor antagonism leads to increased glutamine levels, supporting some of the earlier MRS findings. Also, although not statistically significant, the relationship between increases in glutamine with poorer Stroop performance suggests that disturbances in the glutamatergic system may be responsible for one particular aspect of executive dysfunction, namely response suppression.

Human research also suggests ketamine may impair encoding of nonspatial learning, but not retrieval of nonspatial information already learned (Krystal et al., 2000). By examining order dependency effects of WCST coupled with ketamine administration, Krystal and colleagues (2000) demonstrated that ketamine interferes with initial acquisition (learning) if administered on the first day of testing. However, ketamine does not worsen performance on a second test day when implementation of matching rules has already been established. These results were replicated in another study in which ketamine impaired both spatial and verbal learning, but spared retrieval of information if it was learned prior to drug administration (Rowland et al., 2005a). In a group of 54 healthy controls, ketamine has been shown to impair response inhibition in a cued sentence completion task (Morgan et al., 2004), as well as increase errors of commission on a vigilance task (Krystal et al., 1999a). The above studies suggest a direct role of the NMDA receptor in various aspects of executive functioning including working memory, response inhibition, and attention.

As mentioned, studies have also linked NMDA receptor hypofunction with memory impairment including deficits in delayed recall (Krystal et al., 2005), free recall and recognition (Malhotra et al., 1997), and verbal declarative memory

performance (Newcomer et al., 1999). These studies also highlight the importance of this receptor to efficient memory function, possibly through hippocampal LTP (Malenka and Nicoll, 1999). While the memory impairment suggests temporal lobe involvement, imaging studies using PET and fMRI have shown an association between memory encoding and PFC activation (Fletcher et al., 1998a; Demb et al., 1995). The memory impairments caused by ketamine may thus be related to abnormal PFC function.

In light of the research literature reviewed in the preceding chapters, it is clear that glutamate is involved in the pathophysiology of schizophrenia. Proton MRS has provided an exciting opportunity to examine the glutamate system *in vivo* in both patients and healthy controls. Ketamine studies have implicated the NMDA receptor in various aspects of executive functioning, including response inhibition, working memory, and attention. Given the recent MRS finding of increased Gln following ketamine administration, and its relationship to deficits in response suppression (Rowland et al., 2005b), it is clear that this neuroimaging modality is well suited to studying NMDA receptor hypofunction mediated cognitive dysfunction. No studies to date have demonstrated direct links between executive function and glutamate in a first episode psychosis sample. Only one prior study of anorexia nervosa found executive functioning was associated with Glx levels in the anterior cingulate (Ohrmann et al., 2005a).

B. Materials and Methods

The aim of the current study is to explore the relationship between *in vivo* glutamate levels and executive functioning. Specifically, the Halstead Category Test (HCT) and the Hayling Sentence Completion Test (HST) will be used to measure executive skills. It is hypothesized that: (1) First episode patients (FEP) will have lower levels of glutamate compared to matched healthy controls (HC), consistent with the research literature and the hypoglutamatergic model of schizophrenia. (2) First episode patients will display greater executive impairment compared to controls on the HCT and HST response suppression measures, which are known to be adversely affected in schizophrenia. (3) Glutamate will be related to executive performance such that lower levels will be related to greater executive dysfunction, consistent with studies on the effect of NMDA receptor antagonism on cognition.

Chapter 5

Materials

Clinical Scales

Participants were administered the Structured Clinical Interview for DSM-IV (SCID) (Spitzer et al., 1995) to determine diagnosis in the patient group, and to exclude Axis I pathology in the control group. The Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) was used to quantify negative and positive symptomatology, as well as general psychopathology, in the first episode sample. Each item is rated on a seven point severity scale, ranging from 1 (absent) to 7 (extreme). The possible score range is 7 to 49 on each of the positive and negative symptom sections, and from 16 to 112 on the general psychopathology items.

Two self report measures of psychosis proneness were utilized: the Magical Ideation Scale (MIS) (Eckblad and Chapman, 1983) and the Social Anhedonia Scale (SAS) (Chapman et al., 1976). The MIS is a 30 item questionnaire which asks the subject to label each statement as true or false. It assesses beliefs related to magical thinking (e.g. *the hand motions that strangers make seem to influence me at times*). Higher scores indicate a more schizotypal pattern of responding (Eckblad and Chapman, 1983). The SAS is a 40 item questionnaire which again asks the subject to label each item as true or false. This scales assesses deficits in the ability to derive pleasure from social interactions (e.g. *if given the choice, I would much rather be with others than be alone*). Social anhedonia is presumed to be predictive of psychosis proneness (Mishlove and Chapman, 1985). Norms for these two scales are derived from samples collected by Chmielewski and colleagues (1995).

Within the Edmonton Early Psychosis Intervention Clinic (EEPIC), several additional clinical scales were administered to determine the presence of comorbid psychopathology. These included the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) for the presence of obsessions or compulsions, Beck Depression Inventory (BDI) for depressive symptoms, Beck Anxiety Inventory (BAI) for symptoms of anxiety, and the Bech-Rafaelson Mania Scale (BRMS) for manic symptoms.

Executive Tests

The Hayling Sentence Completion Test (HST) was developed by Burgess and Shallice (1996) to assess the ability to inhibit a prepotent response (Van der Linden et al., 2005). The task consists of two sets of 15 questions comprising Section One and Section Two. In the first section, participants are read aloud a sentence with the last

word missing. The participant is instructed to come up with the missing word to complete the sentence as quickly as possible. The missing word is strongly suggested by the sentence, for example, "*He mailed a letter without a* _____." In the second section, sentences with the last word missing are again read aloud to the participant, who must now generate a word that is completely unconnected to the sentence.

The Hayling Test provides a measure of basic task initiation speed, as well as performance on a response suppression task. The test yields three measures related to executive functioning: sum of the response latencies in the first section (measured in seconds), and two measures of response suppression in the second section, an error score and total response latency (measured in seconds). Section Two produces two types of errors in response suppression. The first is a straightforward completion of the sentence, known as a Category A error. The second is an answer that is closely related to semantic aspects of the sentence, known as a Category B error. Raw error scores from this test will be converted to a z-score based on control norms (Van der Linden et al., 2005).

Subjects with good performance are able to develop a strategy for the response suppression demands of the task (e.g. looking around the room and picking items) and will generate responses unconnected to the sentence. It has been demonstrated that frontal lobe lesions can cause deficits in both response initiation (i.e. resulting in longer response latencies) and in response suppression (i.e. producing more words related to the sentence (Burgess and Shallice, 1996). The selection of this response suppression task has been guided by its activation of the anterior cingulate cortex,

which, as described earlier, has been implicated in neuroimaging and neuropsychological studies of executive dysfunction in schizophrenia.

The Halstead Category Test (HCT) is a commonly used test of executive functioning presumed to tap into aspects of conceptual reasoning, cognitive flexibility, and set shifting (Minassian et al., 2003). The original published version had 208 slides presented on a projector, divided into 7 subtests and was part of the Halstead-Reitan Neuropsychological Battery (Reitan and Wolfson, 1993). The 120 item HCT version used in this study was developed by Gregory, Paul, and Morrison in 1979. This version consists of all 8 items in Subtest I, the first 16 items in Subtest II, and the first 32 items in each of Subtests III, IV, and V. Subtests VI and VII are not administered. Numerous shorter versions of the HCT exist and have been shown to be equivalent to the standard version, which has a considerably longer administration time (Choca et al., 1997).

In the present study, this shortened HCT version was administered by computer with audio feedback. Participants were informed that they would be viewing different geometric figures and designs on the computer monitor which would remind them of a number ranging between 1 and 4. Their task was to determine which number the picture suggested, and then press the corresponding number on the keyboard. A bell sound indicated a hit and a buzzer sound indicated a miss. Using this auditory feedback, subjects determined what the guiding principle was within each subtest. Scores obtained from this computerized version included errors from each of the subtests, a total error score, and response latencies (measured in seconds). The HCT error score from this shortened version was first converted to an estimated HCT

Total score from the original version using the following formula: Total CT Score = 1.486 (short form CT score) + 3.707 (Charter et al., 1997). After the conversion, a standard score was obtained using published norms from the Booklet Category Test (Heaton et al., 2004).

Advantages of using a computerized version of the HCT include error free standardized administration and the collection of reaction time measures. Equivalence has also been established between computerized versions and the standard HCT task (Choca and Morris, 1992). One study has suggested that the computer version may be more difficult than the standard version (e.g. Berger et al., 1994), but this finding has been questioned as the authors did not use a randomized test-retest design (Choca et al., 1997).

The HCT has been reliably used with head injury patients and shown to be a sensitive indicator of brain dysfunction, though it lacks specificity in being able to predict the site of lesion (Reitan and Wolfson, 1993; Choca et al., 1997). Performance on the HCT is more closely related to Performance IQ on the Wechsler Adult Intelligence Scale (WAIS), especially to the Digit Symbol Coding and Block Design subtests (reviewed in Choca et al., 1997). Age and education are also known to be important in predicting error scores, however, no clear gender effects have been determined (Choca et al., 1997).

Authors have also developed additional scales to enable the HCT to be compared to the WCST, for example, deriving values for perseveration, loss of set, and memory (Minassian et al., 2003). Using this approach, the authors were able to determine that the HCT perseveration score was significantly correlated with

perseverative responses from the WCST (Minassian et al., 2003). It has been noted, however, that when the HCT and WCST are administered to the same individual, if the HCT is administered first, the practice effect decreases perseverative and total errors on the WCST (Franzen et al., 1993). Although the HCT and WCST are considered similar, the HCT is a more difficult task and correlations between the two tests are modest at best (reviewed in Choca et al., 1997). One purported reason for the modest correlations is the frontal regions activated by the tasks (Adams et al., 1995).

The Peabody-Picture Vocabulary Test, third edition (PPVT-III) was used to determine premorbid IQ. Additional tests used to derive an executive domain score are the Wisconsin Card Sorting Test (WCST) (Heaton, 1981) and the Stroop Colour-Word Test (Golden, 1976). Specifically, measures of perseverative errors (PE) from the WCST and interference from the Stroop task, which assess cognitive inhibition, were also used in the calculation of an overall executive z-score, along with the standardized total errors from the HCT and errors from Part B of the HST.

Chapter 6

MRS Methods

Voxel Registration

^{1}H-MRS was performed at the Nuclear Magnetic Resonance (NMR) Centre at the University of Alberta Hospital using a 3 Tesla magnet (Magnex Scientific, Concord, Calif.). The magnet was equipped with actively shielded gradients and contained a spectrometer (Surrey Medical Imaging System, Surrey, U.K.) with a quadrature birdcage resonator. A 2x3x3 cm^3 voxel was positioned such that the 2 cm dimension was both centered on, and parallel to, the midline (using transverse and coronal gradient echo image series). Using the central sagittal slice, the voxel was then positioned with its posterior edge touching the corpus callosum and its inferior edge lying along the anterior commissure-posterior commissure (AC-PC) line. While maintaining one corner in contact with the AC-PC line and an edge in contact with the corpus callosum, the voxel was then rotated until the corners of the anterior edge were equidistant from the brain surface (Figure 4).

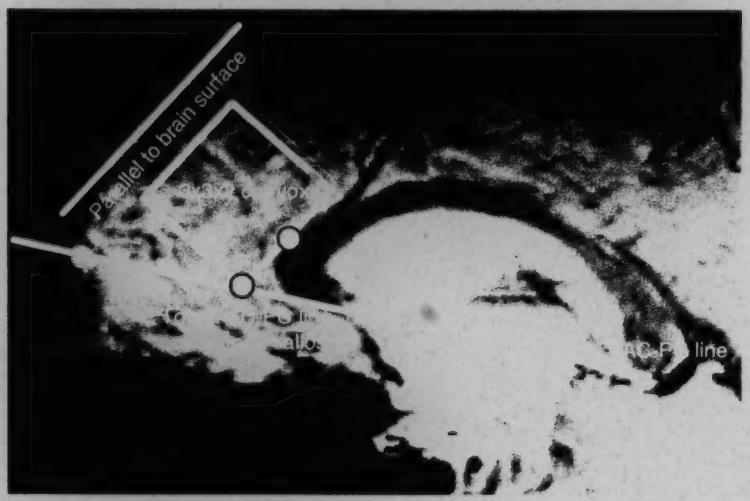


Figure 4. Sagittal view of voxel placement in medial frontal cortex.

Glutamate optimization and in vivo acquisition

Numerical simulation of the response of the central component of the glutamate multiplet at 2.35 ppm to the STEAM TE and TM timings was performed, and compared to the background responses from the other metabolites at a similar chemical shift in the proton spectrum (glutamine, GABA, homocarnosine, NAA, NAAG), with consideration being made for the presence of the macromolecule signal.

In vivo ^{1}H-MRS was then performed for a STEAM registered voxel, following optimization of the magnet field using FASTMAP together with fine tuning of the linear shims. The optimal in vivo glutamate contrast to background was achieved with TE = 240 ms, and TM = 27 ms (TR = 3s, 512 averages) (Figure 5). The long TE time gave minimal macromolecule contamination due to its short T_2 relaxation time, and analysis of the in vivo data using LCModel gave data of equivalent reliability to the singlet resonances from NAA, Cr and Cho. Only metabolites with a fitting error of <20% were used in the analysis.

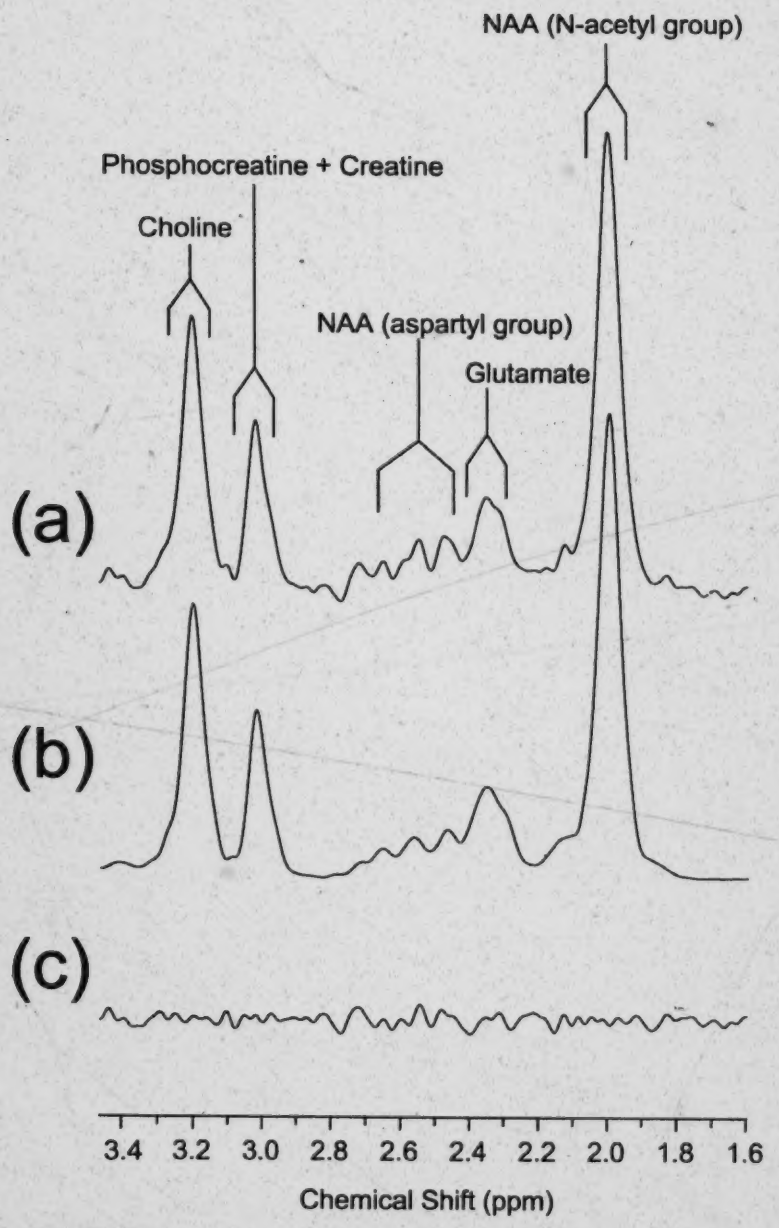


Figure 5. Typical ^{1}H-MR spectrum acquired from a 2x3x3cm^{3} voxel located in the medial frontal cortex, with STEAM timings optimized for glutamate (TE, TM = 240, 27 ms). (a) 2Hz exponential filtered spectrum; (b) LCModel fit spectrum; (c) residual noise following subtraction of spectra in (a) and (b).

Segmentation

Segmentation data from a co-registered voxel was obtained using a modified version of the 1D-projection method we have described previously (Hanstock and Allen, 2000). The modification acquires 10 independent projections of GM, WM or CSF, rather than single data sets. This allows for statistical analysis of the segmentation data from each individual that was not previously possible, without adding greatly to the total time for data acquisition.

Multi-slice gradient echo imaging in the transverse, sagittal, and coronal planes was used to register the PRESS selected volume precisely to the same selected region of brain as that used for the STEAM acquisition. Two hyperbolic secant inversion pulses (110ms length, bandwidth = 150 Hz) were added to the PRESS pulse sequence in which the pulses were 90° sinc-Gauss and 180° optimized-sinc shapes. Prior to the 90° pulse a 15 ms spoiler gradient was applied to dephase any transverse magnetization resulting from the inversion pulses. PRESS parameters used for acquiring 1D projections were: TR = 9 s, TE = 120 ms, 2 averages with 5 kHz sample frequency were digitized over 128 data points. Shimming over the PRESS volume was performed by turning the frequency encode gradient off and increasing the digitization to 2048 data points. Typical shimmed line-width in the frontal region of the brain was < 0.05 ppm.

Previous studies were used to provide estimates of the T_1 values for the three brain compartments of 1070 $\pm$ 60 ms, 720 $\pm$ 30 ms, and 4440 $\pm$ 50 ms for GM, WM and CSF respectively. Using the expression derived by Redpath and Smith (1994), two pairs of T_{inv1} and T_{inv2} timings were computed which gave simultaneous nulls of the

CSF compartment with either that from GM or WM. Ten 1D-projections were acquired for each set of inversion timings, and in each case were the sum of 2 averages. An additional ten 1D-projections were acquired with no inversion pulses and with TE of 500ms. This minimized the signal contamination from GM and WM (< 0.2% residual signal after accounting for T_2 losses) while maintaining significant signal from CSF (~50% residual signal).

Following phase correction of each projection, 3D-surface and contour maps were generated to confirm the selected T_{inv1} and T_{inv2} timings were resulting in simultaneous nulls of CSF with either GM or WM. Subsequently, GM and WM projections were selected from the double inversion recovery 1D-projection series. The T_{inv1} and T_{inv2} timings that were used to acquire these two series of projections for either GM or WM, were then used to estimate a normalization factor which reflected how much each projection had been attenuated due to T_1 and T_2 losses, and therefore, fully account for all of the acquisition timings. Similarly, a normalization factor was estimated for the CSF projection. Segmentation resulted from first normalizing and then summing the signal across each of the three projections such that the relative proportions of GM, WM and CSF could be estimated. All computations necessary for calculating experimental timings prior to acquisition, and for the data analysis, were performed using the MATLAB program environment.

Quantification Data Analysis

The following is a description of the data analysis for the quantification of brain metabolites using water as an internal standard. Initially, three series of data were used for quantification.

1. *Metabolite peak area estimates are extracted from the from the LCModel output (M_{TE240}).*

2. *Segmentation information for GM, WM, and CSF compartment sizes are used to estimate water concentration in the selected brain voxel (W_{brain}).*

3. *Internal water data acquired at different TE values are used as the reference MR signal standard (W_{TE0}).*

The water data were first imported into the processing software, filtered, Fourier transformed, and phase and baseline corrected. The water peak area from each spectrum in the TE series was first determined. These area data were fitted to a multi-exponential using a non-negative-least-squares algorithm, yielding both the T_2 components present in the decay, and their relative proportions. In addition, this permitted an estimation of the water peak area at a theoretical TE of 0 ms (W_{TE0}).

The following formulas were used to derive the metabolite "absolute" concentrations:

Metabolite and water MR signals and concentrations are related by the simple expression:

$$\frac{WaterSignalBrain}{WaterConcBrain} = \frac{MetaboliteSignalBrain}{MetaboliteConcBrain}$$

Rearranging the formula in terms of the metabolite concentration:

$$MetaboliteConcBrain = \frac{(WaterConcBrain * MetaboliteSignalBrain)}{WaterSignalBrain}$$

Defining the term WaterConcBrain:

$$PureWaterConc = 1000 * (1000 / MW_{water})\ mM$$

$$GM_{water} = 0.8 * PureWaterConc$$

$$WM_{water} = 0.65 * PureWaterConc$$

$$WaterConcBrain = W_{brain} = (GM_{segment} * GM_{water}) + (WM_{segment} * WM_{water})$$

Insert measured variables into the rearranged expression above:

$$MetaboliteConcBrain = \frac{(W_{brain} * M_{TE240})}{W_{TE0}}$$

Allowing for different numbers of averages and metabolite T_2 values for metabolite (M) and water (W) acquisitions:

$$SF\ number\ of\ averages = SF_{av} = sqrt\ M_{averages} / sqrt\ W_{averages}$$

$$SF\ for\ metabolite\ T_2 = SF_{MT2} = exp\ (-TE / T_2)$$

$$MetaboliteConcBrain = SF_{T2} * ((W_{brain} * M_{TE240}) / W_{TE0}) / SF_{av}$$

Note that T_2 values for metabolites were assigned based on average literature values for NAA (350 ms), Cr (150 ms) and Cho (310 ms), and estimated for Glu (380 ms), and on expected normal brain concentration values for the GM:WM mix sampled in our studies. Also note that the scaling factor accounting for metabolite T_2 is only to provide numerical values in the mM range, and the same values are applied to all data. This allows comparison to reported data. One could equally well report the data in institutional units where: $MetaboliteConcBrain = ((W_{brain} * M_{TE240}) / W_{TE0}) / SF_{av}$.

Chapter 7

Study Methods

Study Design

Participants were initially contacted by telephone to determine eligibility for the study protocol. Exclusion criteria that extended to both patient and control groups included the presence of ferromagnetic objects in the body (e.g. metal pins, screws, implants, dental hardware), CNS disease, head injury with > 20 minutes loss of consciousness, and any co-existing major medical conditions (e.g. liver or renal insufficiency, significant cardiac, vascular, pulmonary, GI, endocrine, neurological, or metabolic disturbances).

First episode patients (FEP) were recruited from the Edmonton Early Psychosis Intervention Clinic (EEPIC). DSM-IV acceptable diagnoses in the first episode sample included schizophrenia, schizophreniform, substance induced psychosis, brief reactive psychosis and psychosis not otherwise specified. Patients were required to have been ill for less than one year, with a lifetime exposure to antipsychotics not exceeding 3 months total duration. Acceptable age cutoffs were determined to be between 16 and 35 years of age. Exclusion criteria for the patient group included other Axis I DSM-IV disorders, mental retardation, exposure to long-acting depot neuroleptic medication, known sensitivity to olanzapine, risperidone, or quetiapine (patients are randomized to one of these three drugs within EEPIC), clinically significant laboratory or ECG abnormalities, poorly controlled illnesses (diabetes, hypertension), and prior neuroleptic malignant syndrome or allergic reactions to medications.

Healthy control volunteers (HC) were recruited from the local university population via poster advertisement and matched to the patient group as closely as possible on age, education, gender, handedness, and socioeconomic status. Healthy participants were required to have no current or past history of an Axis I psychiatric disorder. Other exclusion criteria were history of substance/ alcohol dependence or abuse, a positive family history of Axis I disorders in first or second degree relatives, and difficulty understanding English.

Prior to study entry, written informed consent was obtained from all participants. After formal consent, subjects were first administered the SCID. With the first episode sample, the SCID was completed by an EEPIC clinician, either at the EEPIC clinic, or in the hospital of admission for inpatients. For healthy volunteers, the SCID was used to rule out any Axis I psychopathology.

Once eligibility was determined, demographic information was collected, and the self report measures (SAS, MIS) were administered. In addition, assessments of overall functioning with the Global Assessment of Functioning scale (GAF) and socioeconomic status (SES) with the Hollingshead scale (Andreasen et al., 1992), were also completed.

Tests of executive function (HCT, HST) were then administered using the standardized protocols outlined in their manuals. For healthy volunteers, two additional tests of executive function, the Wisconsin Card Sorting Test and the Stroop Colour-Word Test, were also administered to match the cognitive battery completed by the first episode patients within the EEPIC clinic.

Following testing, participants were accompanied to the Nuclear Magnetic Resonance (NMR) Centre at the University of Alberta Hospital. Prior to entering the facility, subjects completed a second consent form detailing MRI exclusion criteria as required by standard safety protocols. Specific procedures for the scan were reviewed prior to entry into the scanning room. Participants were asked to lie on their backs on the scanner bed and make themselves as comfortable as possible. A pillow was placed under the knees to minimize back discomfort and a blanket was provided to regulate temperature while inside the scanner. Ear plugs and headphones were also provided to diminish the volume of noise emitted during the scan process. The head coil was placed around the participant's head, with a mirror attached allowing the participant to see outside the scanner. The scans lasted approximately 1 hour and 15 minutes.

Study Subjects

Total subjects that entered the study were 13 patients with a first episode of psychosis and 20 healthy volunteers. Diagnostic stability was assessed within the EEPIC clinic with a repeat SCID at follow-up. Three patients were excluded from the analysis, two for having a follow-up affective diagnosis of Major Depressive Disorder, Severe with Psychotic Features, and one patient for having primarily Axis II pathology. One healthy volunteer did not return for assessment of executive skills and was excluded from the following analyses. The final sample included 10 first episode patients (mean age 22.20 years $\pm$ 3.99) and 19 healthy volunteers (mean age 21.11 years $\pm$ 2.85).

Study Analysis

Demographic and clinical information for the patient and control groups were analyzed using independent t-tests for continuous variables and chi-square tests for categorical data. Between group spectroscopy results using water corrected values and executive performance were analyzed using independent t-tests. In cases where normality and equality of variance assumptions were not met, Mann-Whitney nonparametric between group analyses were used. Relationships between glutamate levels and executive performance were analyzed using Pearson's r for parametric and Spearman's rho for nonparametric correlations. Statistically significant correlations were examined further for their predictive power by entering them into a stepwise regression model using glutamate levels (high/ low) to predict executive performance, after accounting for education and premorbid IQ. Unless otherwise indicated, significance levels were set at $p<0.05$, two-tailed. All statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences) software for Windows®, Version 12.0.

C. Results

Chapter 8

Demographic and Clinical Information

Demographic information for the FEP and HC groups is listed in Table 1.

Group	FEP	HC		
Variable	Mean (SD)	Mean (SD)	test statistic	p-value
Age	22.20 (3.99)	21.11 (2.85)	$t(27)=0.86$	$p=0.40$
Education	12.00 (1.94)	13.95 (1.47)	$t(27)=-3.03$	$p=0.005$
Premorbid IQ	103.2 (7.66)	118.79 (7.86)	$t(27)=-5.12$	$p<0.001$
Gender (M:F)	8:2	17:2	$\chi^2_{(1)}=0.495$	$p=0.48$
Hand (R:L)	8:2	15:4	$\chi^2_{(1)}=0.004$	$p=0.95$
SES			$\chi^2_{(2)}=4.19$	$p=0.12$
Univ/college	2	10		
HS graduate	7	9		
Semi-skilled	1	0		
MIS	8.30 (3.86)	2.79 (2.12)	$U=18.00$	$p<0.001$
SAS	10.20 (4.61)	6.06 (5.80)	$t(26)=1.94$	$p=0.06$

Table 1. Demographic information on the FEP and HC samples.

The groups did not differ significantly in terms of age, however, educational achievement was different between the two groups with the HC participants completing more years of education than the FEP group. Similarly, the HC participants had higher standard scores on the PPVT-III estimated premorbid IQ.

Chi-square analysis for categorical data indicated that the groups did not differ significantly in terms of gender, handedness, or socioeconomic status (SES) distribution. While the HC group was divided socioeconomically between the professional/ advanced degree class (52.6%) and the skilled worker/ high school

graduate class (47.4%), the FEP group belonged predominately to the latter group (70.0%).

Psychosis proneness scales reveal that the first episode sample scored significantly higher on the Magical Ideation Scale compared to the control sample. The patient group had higher scores on the Social Anhedonia Scale but there was only a trend for significance on this measure. The scores reported are in the mild range of severity as cutoff scores for the MIS are 22 for males and 21 for females respectively (Eckblad and Chapman, 1983). Likewise, cutoff scores for the SAS are 20 for males and 16 for females (Mishlove and Chapman, 1985). One HC subject did not complete the SAS.

SCID diagnoses for the patient sample included two individuals with paranoid schizophrenia, one with schizophreniform disorder, one with brief reactive psychosis, and six individuals with substance induced psychosis. Clinical information on the first episode sample is listed in Table 2.

Scale	Range	Mean	(SD)
GAF	30-60	44.78	(9.44)
PANSS Positive	10-25	17.90	(5.93)
PANSS Negative	11-27	18.60	(5.64)
PANSS General	25-50	36.20	(7.57)
BDI	5-24	13.56	(6.52)
BAI	0-28	17.78	(9.22)
BRMS	0-5	2.22	(2.05)
YBOCS Obsessions	0-13	2.33	(4.80)
YBOCS Compulsions	0-5	1.11	(2.21)

Table 2. Clinical scales administered to the first episode sample.

The mean PANSS positive and negative scores are in the mild range of symptom severity. The overall range of scores, however, suggests that some of the first episode patients approached moderate positive and negative symptom severity. The mean BDI (13.56) and BAI (17.78) scores for the sample are in the mild range of severity. The YBOCS and BRMS are negligible in this sample as the mean scores fall in the subclinical range.

Chapter 9

Executive Test Results

Measures of interest from the HST included time taken to complete the task (Part B time – Part A time), and type of errors committed in Part B (Category A errors vs. Category B errors). For the HCT, total error score over the five subtests was used to compare performance between the groups. Total perseverative errors from the WCST and the standardized interference score from the Stroop were also evaluated. Levene's test for equality of variance indicated unequal variances for individual test score measures, with the exception of the standardized Stroop score. Thus, Mann-Whitney non-parametric tests for between group comparisons were used primarily in this analysis. The only exception was the independent t-test used to evaluate Stroop interference.

	Group	FEP	HC	U/t	p-value
Test	Measure	Mean (SD)	Mean (SD)		
HST	B-A time (seconds)	49.33 (43.52)	13.89 (15.76)	36.00	0.02
	Category A errors	3.33 (2.24)	0.89 (1.15)	23.50	0.002
	Category B errors	3.89 (2.71)	1.58 (1.77)	40.50	0.02
HCT	Total Errors	18.60 (11.53)	11.74 (5.63)	59.50	0.10
WCST	Perseverative errors	16.20 (13.66)	6.78 (2.16)	54.50	0.09
Stroop	Interference t-score	54.90 (3.75)	54.22 (8.23)	0.25	0.81

Table 3. Comparison of performance across the four executive tests.

Table 3 summarizes group performance in each of the four executive tasks used in this study. On the HST, patients required more time to complete the task and committed more errors in Section Two of the task. Specifically, the FEP group made more straightforward completions (Category A errors) of the strongly cued sentence and

more semantically related completions (Category B errors) than the HC group. Patient performance on the HCT and WCST did not produce a significantly higher number of errors, although patients performed more poorly on both tasks. Stroop interference also did not differentiate the groups. One HC subject did not complete the Stroop task.

Individual test scores were converted to standardized z-scores based on normative healthy control data as outlined in the Methods section. As indicated, these were based on total errors on Section Two of the HST, total error scaled score from the HCT, perseverative errors on the WCST, and the interference score from the Stroop task. A summary of the z-score comparisons between groups is found in Table 4. Again, standardized performance on the Stroop and HCT was not significantly different between the FEP and HC groups. However, total HST errors and WCST perseverative errors differed significantly.

Z-score	HST	HCT	WCST	Stroop
Group	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
FEP	-1.08 (1.77)	1.96 (0.30)	-0.84 (1.82)	-0.49 (0.38)
HC	0.92 (1.07)	1.77 (0.57)	0.45 (0.33)	-0.42 (0.82)
p-value	0.001[a]	0.12[a]	0.04[b]	0.81[a]

[a]independent t-test, [b]Mann-Whitney non parametric test

Table 4. Z-score comparison between groups across the four executive tests.

The overall executive z-score (zexe) was calculated by taking the average of the four z-scores. Comparing zexe across groups, the HC group performed significantly better than the FEP group (0.30 ± 0.32 vs. -0.71 ± 0.84 respectively, $U=17.00$, $p=0.001$). In light of the differences in education and premorbid IQ between

the groups, it was necessary to evaluate if the study variables were related to these two factors. Premorbid IQ was positively correlated with the executive z-score ($r=0.67$, $p<0.001$) in the total sample, but education was not ($r=0.11$, $p=0.59$). When stratified by group, IQ was not related to zexe in the FEP group ($r=0.12$, $p=0.76$), but was positively correlated in the HC group ($r=0.72$, $p=0.001$). In both groups, education was not related to premorbid IQ (FEP: $r=-0.41$, $p=0.27$, HC: $r=-0.40$, $p=0.10$).

Chapter 10

MRS and Segmentation Results

Levels of NAA, Cho, Glu, and Cre were compared in the FEP and HC groups. Figure 6 shows between group comparisons for each of the four main metabolites.

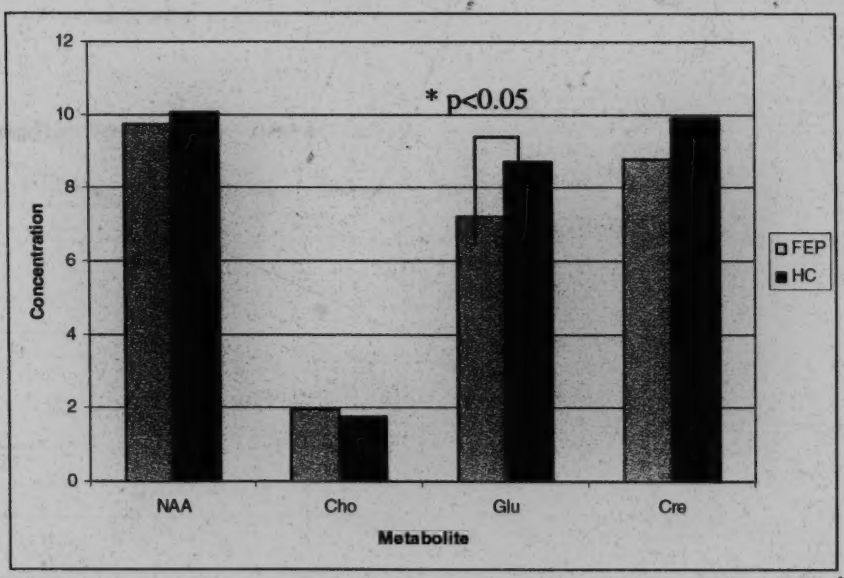


Figure 6. Between group comparison of metabolite levels measured by ^{1}H-MRS.

As seen in Figure 6, levels of NAA, Glu, and Cre were lower in the FEP group, but only Glu reached statistical significance. Cho, although higher in the FEP group, was not significantly different from the HC group.

Table 5 summarizes mean metabolite concentrations for each group. Glu was predicted to be lower in the FEP group and consequently, a one-tailed test of significance was applied for this metabolite. NAA was also predicted to be lower in the FEP group, consistent with the research literature, and thus, a one-tailed test of significance was applied to this metabolite as well. Two-tailed tests of significance were used for the remaining metabolites.

Metabolite	NAA	Cho	Glu	Cre
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
FEP	9.72 (1.26)	1.96 (0.30)	7.21 (1.28)	8.78 (1.92)
HC	10.06 (1.29)	1.77 (0.57)	8.69 (2.23)	9.93 (2.00)
t	-0.67	0.96	-1.92	-1.49
df	27	27	27	27
p-value	0.25	0.35	0.03	0.15

Table 5. Metabolite concentrations in FEP and HC groups.

An examination of the boxplot for Glu levels by group (Figure 7) shows a restricted range of values for the FEP group.

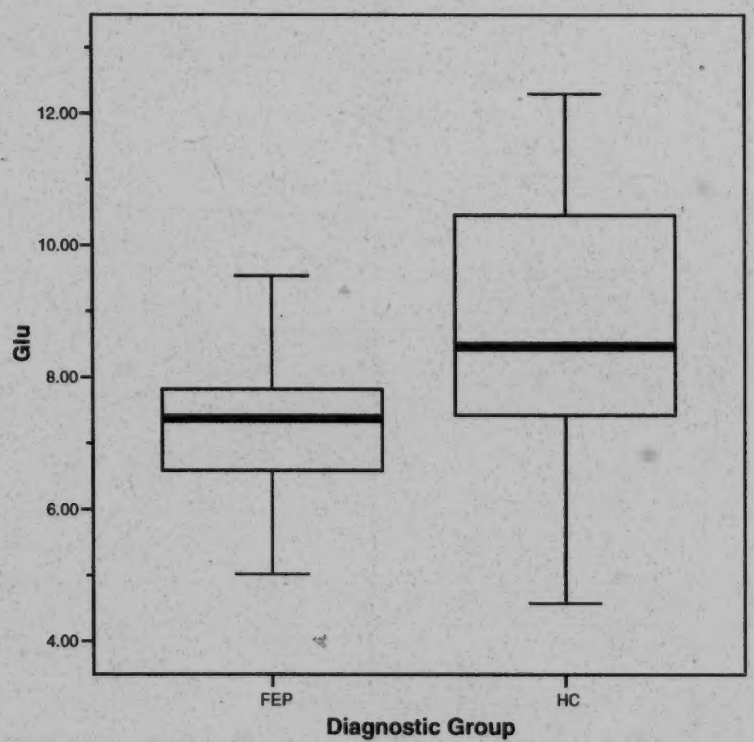


Figure 7. Boxplot of glutamate (Glu) levels in FEP and HC groups.

In order to determine how the patient glutamate values compared to the HC group, a median split was applied based on the normal control group yielding a cutoff glutamate value of 8.47 to divide participants into high versus low glutamate categories. Since one person landed on the median, values <8.48 were grouped in the low glutamate category, and values >8.47 in the high glutamate category. Combining across gender, 90% of patients fall into the low glutamate group, $\chi^2_{(1)}=4.05$, p=0.04. Larger sample sizes would be needed to see if this relationship provides any predictive power in terms of diagnosis.

Segmentation data from the total sample showed an overall GM: WM ratio of 69.1%: 30.9%. When stratified by group, the two samples did not differ in voxel composition with the FEP group having 70.3% GM to 29.7% WM compared to the HC group with 68.4% GM to 31.6% WM. The water quantified metabolite values presented above correct for segmentation.

As Glu was the only significant metabolite result, an examination of Glu distribution as a function of tissue composition was undertaken. Glu was not significantly correlated with GM/GM+WM in the total sample (r=0.27, p=0.15). Figure 8 shows the relative distribution of Glu as GM increases in each group separately.

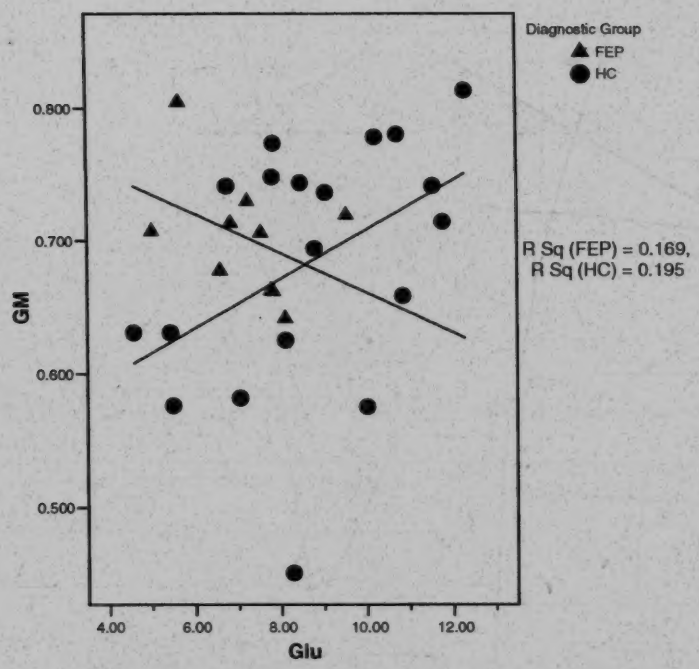


Figure 8. Scatterplot of glutamate (Glu) versus grey matter (GM) in FEP and HC.

Although not statistically significant in either group (HC: r=0.44, p=0.06, FEP: r=-0.41, p=0.24), the HC group shows an increase in Glu as GM increases, but an inverse relationship is seen in the FEP group. This finding could be an artifact of the smaller sample size and restricted ranges in the FEP group. An examination of the scatterplot shows that with a larger sample, the few outliers driving the negative correlation may change the direction of this relationship.

Chapter 11

Relationship Between MRS and Executive Function

Correlational analysis assessing the role of premorbid IQ and education was repeated on the MRS variables. Education was not related to any of the four metabolites, but premorbid IQ was inversely related to choline (r=-0.39, p=0.04) in the total sample. None of the other metabolites were related to premorbid IQ in the total sample (NAA: r=-0.20, p=0.30; Glu: r=-0.05, p=0.80; Cre: r=0.01, p=0.94). Table 6 shows a correlational matrix for each of the four metabolites against education and premorbid IQ separated by group. The significant correlations seem driven by the larger HC group.

	Metabolite	NAA		Cho		Glu		Cre	
Group		R	p-value	R	p-value	R	p-value	R	p-value
FEP (n=10)	PPVT-III	-0.29	0.41	0.12	0.74	-0.18	0.61	0.16	0.66
	Education	-0.15	0.68	0.60	0.07	0.21	0.57	0.27	0.44
HC (n=19)	PPVT-III	-0.46	0.05	-0.52	0.02	-0.52	0.02	-0.46	0.05
	Education	0.35	0.15	0.11	0.64	0.05	0.85	0.01	0.96

Table 6. Correlational matrix of metabolite levels with premorbid IQ and education.

No apparent relationship exists between educational achievement and metabolite levels in either group. Significant negative correlations are seen between metabolite levels and premorbid IQ in the HC group, but not the FEP group. Again, this may be due to the greater variance in the HC group, as no significant correlations between

metabolites and premorbid IQ is seen in the total group, with the exception of the negative correlation with choline.

To examine the possible relationship between glutamate levels and executive performance, correlational analysis was undertaken using Spearman's rho (σ) for non-normal distributions. As seen in Figure 9, no relationship exists between the overall executive z-score and the glutamate values obtained (σ=0.007, p=0.97). The lack of any significant correlations precludes the use of regression analysis with this metabolite.

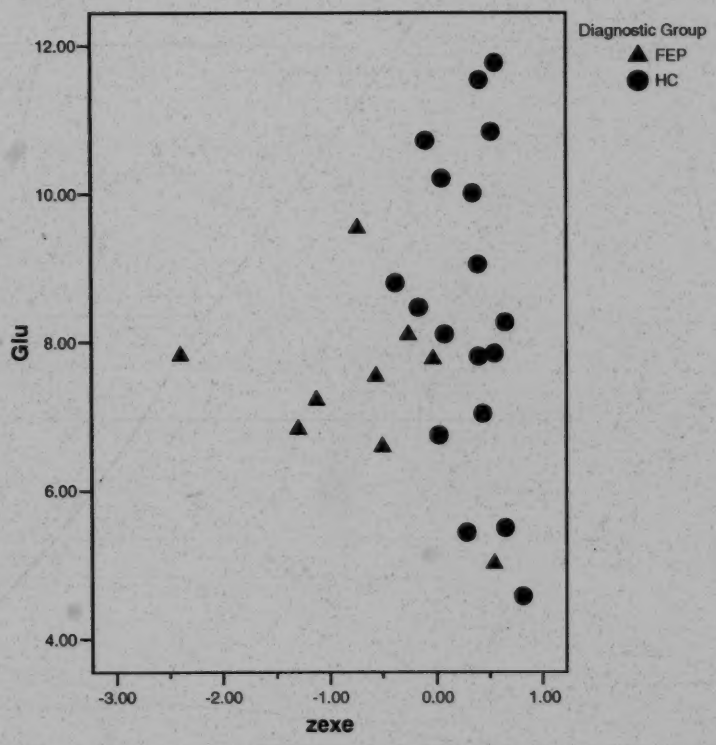


Figure 9. Scatterplot of executive z-score (zexe) and glutamate (Glu) concentration.

No other metabolite was related to zexe in the total sample (Table 7). When divided by group, Cho and Cre were inversely related to zexe in the HC sample.

Metabolite		NAA		Cho		Glu		Cre	
		rho	p-value	rho	p-value	rho	p-value	rho	p-value
Total group	zexe	-0.16	0.44	-0.32	0.12	0.007	0.97	-0.23	0.25
FEP	zexe	-0.03	0.95	0.18	0.64	-0.23	0.55	-0.06	0.88
HC	zexe	-0.43	0.07	-0.48	0.04	-0.18	0.48	-0.52	0.03

Table 7. Correlational matrix of metabolite levels with overall executive z-score.

Again, the lack of statistically significant correlations between the other metabolites and zexe in the total sample precludes further analysis using regression models.

D. Discussion

The study aim was to examine the role of glutamate in executive functioning in unmedicated first episode psychosis patients and healthy controls. Consistent with the first study hypothesis, lower glutamate levels were seen in the FEP group. This finding lends additional evidence to the research literature supporting a hypoglutamatergic model of the illness. MRS studies reporting Glu or Glx reductions in schizophrenia have only been seen in chronic patients thus far (Ohrmann, et al., 2005b; Théberge et al., 2003). This is the first study to report reduced Glu in a first episode sample. Bartha and colleagues (1997) found significant increases in Gln in the left medial PFC in their sample of first episode patients. This finding was replicated by Théberge and colleagues (2002) who similarly found increased Gln in the left anterior cingulate and thalamus in a first episode sample. However, both studies failed to find direct decreases in Glu, and have argued that if the primary pathology lies in dysfunctional Gln-Glu cycling, the overall cellular Glu would be unchanged (Bartha et al., 1997). The current 3T study unfortunately could not obtain reliable Gln values (fitting error of <20%) and therefore, could not replicate these reported increased Gln findings.

Increased Gln levels have been interpreted in two ways. The first interpretation suggests that higher Gln indicates a hyperglutamatergic state as the excess Gln is converted directly to Glu (Rothmann et al., 1999). The second interpretation posits that increased Gln levels indicate a hypoglutamatergic state if there is a dysfunction in the conversion of the available Gln to Glu (Bartha et al.,

1997). The current finding of decreased Glu in a first episode sample provides additional evidence for the second interpretation.

What remains uncertain is the timing and course of glutamatergic abnormalities in the illness. Elevated glutamate/ glutamine (Glx) in a sample of adolescents at high genetic risk for schizophrenia (Tibbo et al., 2004) lends support to a potentially excitotoxic process leading to the diminished Glu levels seen in chronic samples (Ohrmann et al., 2005b; Théberge et al., 2003). However, the current finding of decreased Glu in a first episode sample suggests a hypoglutamatergic state exists early in the illness process. This argues against the notion of Glu reductions in a chronic group as being the result of illness duration or medication use (Théberge et al., 2003), but may be more related to the core pathophysiology of the disorder.

A single MRS scan, however, provides only a snapshot of a dynamic system. Changes in glutamate levels in either direction can occur in response to a variety of factors. For example, NMDA receptor hypofunction (using receptor antagonist models in preclinical studies) may initially increase release of glutamate (Liu and Moghaddam, 1995), but this may eventually lead to a compensatory downregulation of receptors and/ or neurotransmitter function in response to excess glutamatergic neurotransmission at non-NMDA receptors (AMPA/ kainate) (Moghaddam et al., 1997). Since MRS is unable to differentiate between the compartments of the CNS, differences in Glu levels seen between patient and control groups need to be interpreted cautiously. As seen with studies examining glutamate synthesis and metabolism (Burbaeva et al., 2003; Gluck et al., 2002), difficulties arise in determining where the initial pathology occurs in the system.

Methodological advantages of this study over some of the earlier MRS work may have allowed for better quantification of glutamate. For instance, the use of a higher field strength magnet allowed the separation of the Glu peak from the Glx (Glu+Gln+GABA) complex. Additonally, the glutamate optimized pulse sequence provided equivalent reliability for Glu compared to the other singlet resonances (e.g. NAA). Also, acquiring segmentation data ensured equal GM:WM mixture between groups for more accurate comparisons of metabolite levels in the selected voxel. Finally, quantification of the metabolites using water as an internal standard rather than reporting the results as ratios to other metabolites prevented changes in the value of the denominator in the ratio from adversely affecting the results.

Initial analysis of executive performance found executive cognitive deficits in the patient sample consistent with the second study hypothesis. The FEP group had longer response latencies and made more errors of response suppression on the HST. This result suggests deficits in cognitive inhibition, which implicates abnormal anterior cingulate function. Other studies have found abnormal anterior cingulate metabolism related to another response suppression measure, Stroop incongruent errors (Carter et al., 1997; Nordahl et al., 2001). However, no differences in performance were seen on the Stroop task in this study. This was an unexpected finding as response suppression requirements are similar between the Stroop and the HST. In the Stroop task, subjects must suppress the reading response in favour of naming the ink colour (e.g. for the stimulus **red**, subjects must say "blue" rather than "red"). The slowing of this response compared to the first two conditions (simply reading words or colour naming without incongruent stimuli) comprises the

interference score. This is similar to suppressing the expected sentence completion response in Section Two of the HST. One possible reason for the discrepancy in performance between these two tasks could be the norms applied to the HST. These were derived from Van der Linden and colleagues (2005) study based on 16 healthy controls. A larger normative sample that better matched for age, gender, and IQ of the current sample might have adjusted for differences in performance more accurately.

Patients did not perform significantly worse on the HCT as was expected, although more perseverative errors were made on the WCST consistent with the literature on the neuropsychology of schizophrenia. Conceptual set shifting ability, necessary for the performance of the WCST, is comparable to the task demands of the HCT, which is generally considered to be a more difficult task (Choca et al., 1997). In the current study, patients made significantly more perseverative errors on the WCST, but not on the HCT. The increased difficulty and complexity of the HCT may be one reason why the HC group performed similarly to the FEP group. Both groups might have found the task similarly challenging. Despite the lack of statistically different performance on the HCT, patients still made more errors on this test (FEP: 18.60 ± 11.53 vs HC: 11.74 ± 5.63).

An inability to determine the new basis for categorization in the HCT may also represent difficulty suppressing categorization demands of an earlier subset. Similar logic has been applied to the WCST in which perseverative errors are viewed as the inability to suppress an earlier successfully applied sorting criterion, i.e. defective inhibitory mechanisms (Morgan et al., 2004). While the response suppression demands of the HST and Stroop were originally being contrasted with the higher order

conceptual set shifting skills required for the WCST and HCT, the above explanation would suggest that these tests may actually reflect similar response inhibition processes. In which case, studies implicating the NMDA receptor system in executive dysfunction, specifically in terms of response suppression (Morgan et al., 2004; Rowland et al., 2005b), would lead one to expect an inverse relationship between *in vivo* glutamate levels and executive performance.

The final step, therefore, was to examine whether there was a relationship between *in vivo* glutamate levels and executive functioning, as no other direct associations have been reported in a clinical sample. The third study hypothesis postulated that lower levels of glutamate would be related to greater executive dysfunction, consistent with the literature on the effect of NMDA receptor antagonism on cognition. However, this study failed to find the expected relationship between *in vivo* glutamate levels and overall executive functioning.

There are several possible reasons for this negative finding. Preclinical studies show that ketamine administration has a biphasic effect on Glu efflux in the PFC; specifically, low doses of ketamine may increase Glu levels while higher doses may decrease these levels (Moghaddam et al., 1997). Although it is problematic to make direct comparisons of animal studies as models of illness, this finding suggests that the degree of NMDA receptor hypofunction will affect Glu levels, and consequently, its effect on cognition. Specifically, animal models suggest that Glu, acting through NMDA receptors on various systems, including GABAergic and noradrenergic neurons, maintains inhibitory control over excitatory pathways (Farber, 2003). NMDA receptor antagonism would therefore abolish this inhibitory control over

excitatory inputs, causing disruption of multiple intracellular signaling mechanisms and the eventual impairment of cognitive functions subserved by the affected neurons (Farber 2003). It is difficult to determine the degree of NMDA receptor associated hypofunction present in the current sample. Unlike direct challenge studies, Glu levels are being used here as a proxy measure to infer receptor dysfunction. While the clinical studies of ketamine link NMDA receptor antagonism to specific cognitive deficits (Malhotra et al., 1997; Newcomer et al., 1999; Krystal et al,. 1999a), the nature of the relationship between cognition and glutamate itself in human studies is still relatively unclear.

Restricted ranges in the current FEP group, non-normal distribution of the data, and unequal variances between groups precluded the use of parametric tests in some instances, limiting the statistical power to detect significant relationships. The description of the FEP group also showed sample heterogeneity in terms of diagnosis. Including schizophrenia spectrum disorders with substance induced psychoses may potentially mask different neurobiological mechanisms underlying these two distinct groups. Although it is difficult to determine whether substance use actually triggered the first psychotic episode in some of these patients, the presence of substance use at the onset of symptoms prohibits exclusion of a substance induced disorder, per DSM-IV criteria. Further, although the two groups did not differ significantly on demographic characteristics, education and premorbid IQ was higher in the HC group. This discrepancy is due in part to recruitment of the HC group from the local university population. Although patients performed more poorly on the executive tests, because the overall executive z-score was related to premorbid IQ, this may

explain the group differences. Education was also significantly different between the FEP and HC groups, but was not related to the executive z-score, so it is unlikely to be a contributing factor. Given that psychosis typically begins in the late teens to early twenties, educational and vocational achievement in this group may be lower (Johnson-Selfridge and Zalewski, 2001). An improved recruitment strategy would be to focus on HC participants who have only completed high school and worked in trade/ service oriented careers rather than professional careers.

Another possible reason for the lack of relationship between glutamate levels and executive function in this study may be due to the location of the voxel. The selected voxel targeted the medial PFC and anterior cingulate regions primarily. While the anterior cingulate is believed to be involved with monitoring errors and response suppression demands of tasks such as the Stroop (Carter et al., 1998) and HST, more complicated conceptual set shifting ability required of the WCST additionally activates the DLPFC (Weinberger et al., 1986; Berman et al., 1995). The DLPFC is not localized exclusively in the selected voxel, but contributions from this region cannot be ruled out. While it has been suggested that it is impossible to dissociate the anterior cingulate circuit from the dorsolateral prefrontal circuit (Saint-Cyr et al., 2002), the effects of these different regions may cancel out specific relationships. Further, the voxel was placed over midline which may have potentially cancelled any lateralized differences in glutamate, which have been reported by other authors (Kegeles et al., 2000).

Study Limitations

Several limitations of the present study need to be addressed. Small sample sizes warrant cautious interpretation of the correlational data. Significant statistical correlations in the HC group, but not in the FEP group, could be accounted for by the greater variance in the controls. While the study was sufficiently powered to examine spectroscopy data, larger samples are needed to explore correlations with neuropsychological tests. Further, ideally both groups would have equal sample sizes and equal variances to meet the assumptions required for parametric statistical tests.

Some authors have argued that comparing performance between patients and healthy controls where test performance is not equivalent unfairly biases against patients (Gold and Harvey, 1993). However, among the four tests chosen for this study, patients performed comparably on the HCT and the Stroop. This suggests an adequate ability to perform the demands of these two tasks. Additionally, on the remaining two tests where performance was significantly lower in the FEP group (HST, WCST), an examination of the z-scores obtained are approximately one standard deviation below the mean of normal control data which still falls within the normal range of performance.

Executive function, as mentioned, encompasses numerous sub-processes including attention, working memory, and response inhibition which work in an integrated fashion to perform complex tasks (Funahashi, 2001). While the tasks chosen for this study focused on response suppression components, it is difficult to isolate this function from other related executive processes. In the study of executive function, different tests are sometimes used to assess the same cognitive domain even

though they appear to tap different processes. For example, listing the WCST alongside the Stroop task under the domain of executive functioning fails to capture the differential emphasis each task places on the ability to shift cognitive set and suppress prepotent responses. Likewise, there is also a considerable overlap in executive sup-processes within one task. For example, WCST performance also requires intact working memory and sustained attention for successful task completion. This highlights the difficulty in both operationally defining, and in turn, measuring, a single executive function.

Executive functions also engage diverse cortical networks as described earlier. Perhaps, employing a technique such as spectroscoping imaging (MRSI) which uses multiple voxels to cover numerous brain regions would confer a better "hit" in capturing glutamate's response to executive functioning, rather than on relying on a single voxel in a prescribed region. The smaller voxels needed for these comparisons, however, would sacrifice the signal to noise ratio.

Hopefully, the above discussion does not discourage the examination of the neurochemical underpinnings of executive function. While executive tests tap into multiple processes, making it difficult to implicate a specific neurotransmitter, such as glutamate, to a specific cognitive construct, such as response suppression, it remains a worthwhile endeavour, albeit not an easy one. Using "real time" experiments such as the one described by Rowland and colleagues (2005b), allows for the exciting opportunity to link specific neurochemical mechanisms (NMDA receptor antagonism with ketamine administration) with specific behavioural measures (Stroop

performance) to discrete brain areas (anterior cingulate glutamine levels), and perhaps, represents the future of this area of inquiry.

E. Summary

Considering the role glutamate may play in the etiology and pathophysiology of schizophrenia, it was important to determine whether glutamate was related to the observed executive dysfunction seen in this illness. Thus, the current study attempted to identify the neurochemical correlates underlying executive functioning in first episode psychosis patients and healthy controls. Drug-naïve first episode patients and healthy volunteers underwent one 3T ^{1}H-MRS investigation of *in vivo* glutamate levels and neuropsychological testing of executive functioning. Consistent with the study hypotheses, patients exhibited decreased levels of glutamate in the medial frontal cortex compared to healthy controls, and demonstrated greater impairment on tests of executive functioning. It was also hypothesized that glutamate would be related to executive performance such that lower levels of glutamate would be related to greater cognitive impairment. However, contrary to this hypothesis, no relationship was observed between glutamate levels and executive performance in the patients or in the controls. Considering the degree of functional disability associated with schizophrenia, learning more about the neurochemical aspects of executive dysfunction in this devastating illness remains an exciting and worthwhile endeavour. Larger sample sizes, better matched groups, and more sensitive tests will hopefully shed light on the neurochemistry subserving the most complicated of cognitive processes.

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